LEVELS OF PENTRAXIN-3 IN GINGIVAL CREVICULAR FLUID OF PATIENTS WITH AGGRESSIVE AND CHRONIC PERIODONTITIS- AN IN VIVO STUDY

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfilment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH II
DEPARTMENT OF PERIODONTICS

MAY 2018

CERTIFICATE

This is to certify that this dissertation titled **"LEVELS OF PENTRAXIN-3 IN GINGIVAL CREVICULAR FLUID OF PATIENTS WITH AGGRESSIVE AND CHRONIC PERIODONTITIS- AN IN VIVO STUDY"** is a bonafide record of work done by **Dr. RITIKA CHHALANI** under our guidance and to our satisfaction, during her postgraduate study period of 2015-2018.

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PLACE OF STUDY	**SRI RAMAKRISHNA DENTAL COLLEGE AND HOSPITAL, COIMBATORE-641006.**
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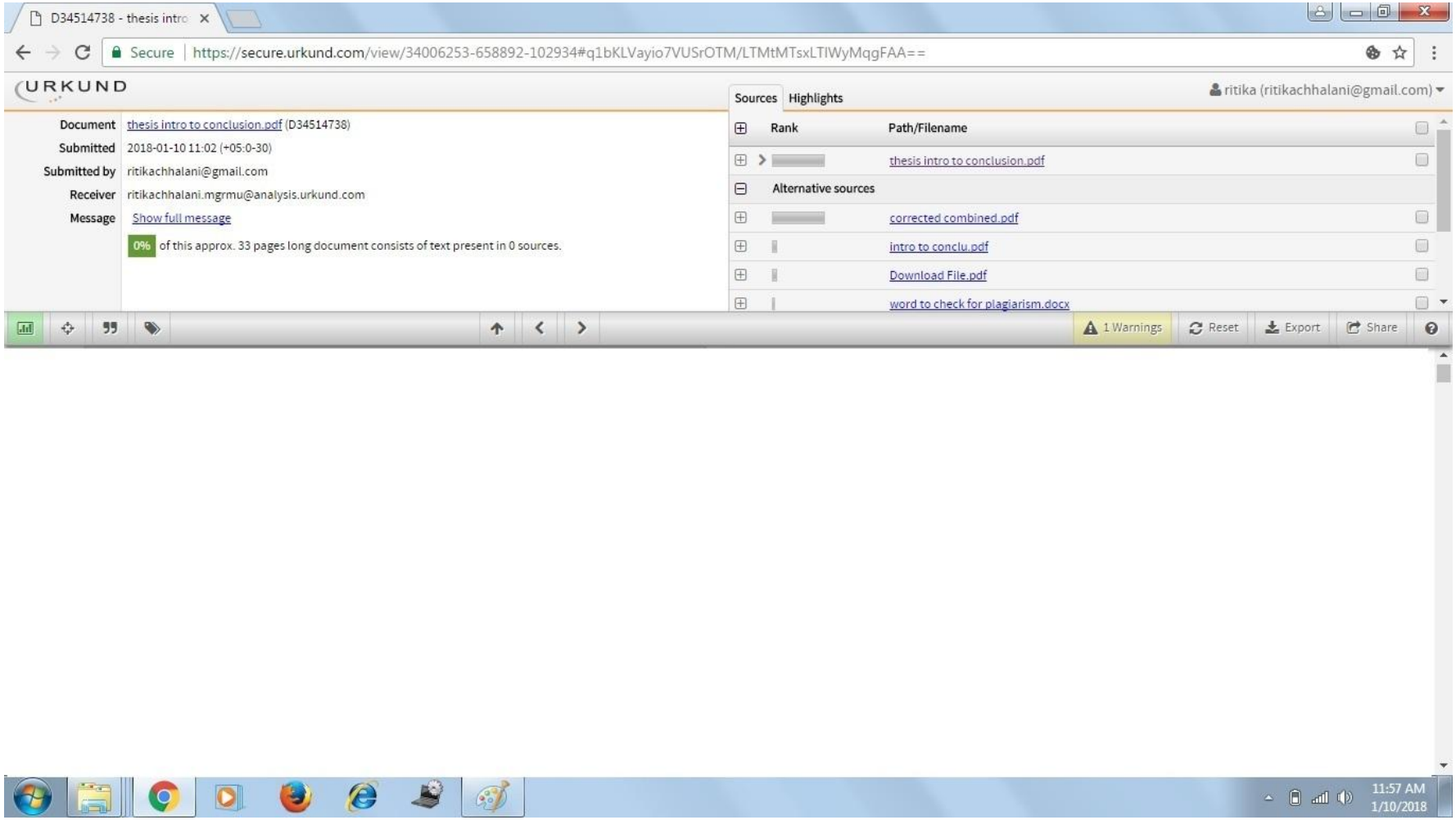
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-Dr. RITIKA CHHALANI

ABSTRACT

Background: Periodontitis is a multifactorial disease and is a major factor for tooth loss. The pathogenesis of this disease has varied and evolved over the decades. It is now believed that the host has a major role to play in the progression of periodontal destruction. The changes that occur in the periodontium are often hard to explain clinically and require an understanding at the biochemical level. Biomarkers, such as acute phase proteins have the potential to shed more light on the changes occurring within the tissues and are believed to be more reliable. Pentraxin-3 is one such acute phase protein.

Aim & Objectives: The aim of this study was to estimate and compare the levels of Pentraxin-3 in the gingival crevicular fluid of aggressive periodontitis, chronic periodontitis and healthy subjects.

Materials & methods: A total of 45 subjects were enrolled in this cross-sectional study. The study groups included subjects with chronic periodontitis (n = 15), those with aggressive periodontitis (n = 15) and those who acted as healthy controls (n = 15). Following the assessment of the clinical parameters, sites with the greatest mean sulcular bleeding index score were selected in every subject to serve as a source of gingival crevicular fluid collection. Gingival crevicular fluid was collected using microcapillary pipettes and processed to estimate Pentraxin-3 levels using enzyme linked immunosorbent assay.

Results: The values obtained were statistically compared. Overall comparisons were made using one way analysis of variance, pairwise comparisons were made using Tukey HSD and overall correlation was assessed by means of Pearson correlation. The values of Pentraxin-3 were the highest in aggressive periodontitis group followed

by chronic periodontitis groups and were the least in the healthy subjects. This overall difference between the groups was found to be of statistical significance. On pairwise comparison, the levels of Pentraxin-3 were higher in the aggressive periodontitis group than in the chronic periodontitis group (mean difference: 4.53ng/mL), in the aggressive periodontitis group than in the healthy group (mean difference: 6.01ng/mL) in the chronic periodontitis group than in the healthy group (mean difference: 1.48ng/mL) with the difference between the groups being statistically significant on each of these comparisons ($p < 0.001$). Overall, a positive correlation was observed between Pentraxin-3 and full mouth probing depth ($r = 0.708$), Pentraxin-3 and individual probing depth ($r = 0.704$), Pentraxin-3 and full mouth clinical attachment level ($r = 0.687$), Pentraxin-3 and individual clinical attachment level ($r = 0.643$), Pentraxin-3 and full mouth plaque index ($r = 0.495$), Pentraxin-3 and mean sulcular bleeding index ($r = 0.752$) and these correlations were found to be statistically significant in each case ($p < 0.001$).

Conclusion: The results obtained from the present study suggest that the levels of Pentraxin-3 rise with increase in activity and severity of periodontal disease. Hence Pentraxin-3 may serve as a valuable biomarker and diagnostic tool in the future.

Key words: Pentraxin-3; gingival crevicular fluid; inflammation; biomarker; chronic periodontitis; aggressive periodontitis.

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LIST OF ABBREVIATIONS

Abbreviation	**Expansion**
AgP	Aggressive Periodontitis
ANOVA	Analysis of Variance
CAL	Clinical Attachment Level/ Loss
CP	Chronic Periodontitis
CRP	C- Reactive Protein
DNA	Deoxyribonucleic Acid
ECM	Extra Cellular Matrix
ELISA	Enzyme Linked Immuno Sorbent Assay
fCAL	Full mouth Clinical Attachment Level/Loss
FGF	Fibroblast Growth Factor
FGFR	Fibroblast Growth Factor Receptor
FH	Factor H
fPD	Full mouth Probing Depth
fPI	Full mouth Plaque Index
GCF	Gingival Crevicular Fluid
H	Healthy
Hs-CRP	High sensitive- C- reactive protein
iCAL	Individual Clinical Attachment Level/Loss
IL	Interleukin
IL-1R	Interleukin 1 Receptor Antagonist

iPD	Individual Probing Depth
iPI	Individual Plaque Index
LBP	Lipopolysaccharide Binding Protein
LPS	Lipopolysaccharide
MBL	Mannose Binding Lectin
MMP	Matrix metalloproteinases
mSBI	Mean Sulcular bleeding Index
Narp	Neuronal Activity Regulated Protein
NP	Neuronal Pentraxin
NPR	Neuronal Pentraxin Receptor
OmP	Outer Membrane Protein
OSAS	Obstructive Sleep Apnea Syndrome
PAD	Peripheral Artery Disease
PI	Plaque Index
PTX	Pentraxin
SAP	Serum Amyloid Protein
SLE	Systemic Lupus Erythematosus
SRP	Scaling and Root Planing
TLR	Toll Like Receptor
TNF	Tumor Necrosis Factor
TSG6	Tumor Necrosis Factor-α induced protein 6
TSG-14	Tumor necrosis factor-inducible gene-14 protein

Inflammation is a major component of periodontal disease, although, its exact role has been a subject of much debate. In recent years, the focus has shifted drastically towards host response, thereby, implicating inflammation among the prime culprits in periodontal disease.

Once the host encounters bacterial or other infectious agents, a variety of chemokines and cytokines are produced at the site. Virulence factors such as, bacterial lipopolysaccharide (LPS) activate numerous pro-inflammatory signals as well as cells of the innate defence that in turn result in the production of a variety of acute phase proteins and cytokines.

Acute phase proteins are those, whose plasma concentrations are altered (increased/decreased) by at least 25% during inflammation.[1] Pentraxin-3 (PTX-3), also known as tumor necrosis factor- inducible gene-14 protein (TSG-14), is one such acute phase protein.

Diagnosis of periodontal disease has traditionally relied on methods such as; visual examination, assessment of probing pocket depth, level of clinical attachment, bleeding on probing and radiographic methods to assess bone loss. All of these methods are limited by the fact that, they require a period of time to pass before the features of disease can be picked up by the clinician.

A diagnostic tool that can pick up disease activity before obvious signs become discernible would hence be of great value. The drawbacks encountered with the traditional techniques, along with the need for quicker assessment to help in damage

control, has led to extensive research being directed towards the diagnostic arena. Much of this research has focused on the search for an ideal biomarker, which could be of diagnostic as well as of prognostic value.

Acute phase proteins, such as PTXs possess ideal properties that make them candidate diagnostic biomarkers.

Pentraxin derives its name from the Greek words; 'Penta', meaning five and 'Ragos', meaning berries, due to its structure. PTXs are a family of evolutionarily conserved proteins and can be short or long. C-reactive protein (CRP) and Serum Amyloid Protein (SAP) are examples of short PTXs while, PTX-3 and -4 are examples of long PTXs. Unlike CRP and SAP that are produced in the liver, PTX-3 is produced by a number of cells such as; leukocytes, fibroblasts, dendritic cells, monocytes, macrophages, epithelial cells, endothelial cells and vascular smooth muscle cells, in response to pro-inflammatory stimuli.

The extra-hepatic synthesis of PTX-3, renders it additional value and it has been suggested to be a true indicator of disease activity.

AIM: The aim of this study was to assess and compare the levels of PTX-3 in gingival crevicular fluid (GCF) and to determine if patients with chronic periodontitis (CP) and aggressive periodontitis (AgP) demonstrate any variations in the same.

OBJECTIVES:

1. To measure and compare the levels of PTX-3 in GCF from patients with AgP and CP
2. To compare the GCF PTX-3 levels in healthy (H) and periodontitis affected patients.
3. To correlate the GCF PTX-3 levels with clinical periodontal parameters.

EFFECTS OF INFLAMMATION ON THE PERIODONTIUM:

Periodontal disease occurs as a result of complex interactions between the subgingival biofilm and the immune-inflammatory events that develop in the gingival and periodontal tissues in response to bacterial challenge. Periodontitis is predominantly an inflammatory disease of the periodontium and hence understanding the impact of inflammation on the periodontal tissues may provide a better understanding about disease progression.

Although gingivitis precedes periodontitis, not all cases of gingivitis progress to periodontitis. Inflammatory changes in the periodontal ligament and alveolar bone result in breakdown of the periodontal ligament fibers, resulting in loss of attachment, along with resorption of the alveolar bone.

Inflammation is a series of highly orchestrated events that affects susceptible tissues including the periodontium. Under normal conditions, this process of inflammation enters a cycle of programmed resolution and activates various pathways that promote healing.[2]

Bacterial plaque was thought to be the primary causative factor of periodontitis in the 1970s and 1980s. At that time it was believed that, poor oral hygiene and increased plaque accumulation, result in periodontal disease. This model however, failed to account for observations such as; many individuals with poor oral hygiene who do not develop advanced periodontal disease, while on the other hand, individuals, despite maintaining good oral hygiene experienced progressive periodontal breakdown as in case of AgP. These findings were supported by the work done by **Löe *et al*. (1986)** on tea laborers in Sri Lanka, who had no access to dental care. These subjects were divided into three main groups: (1)

subjects (approx. 8% of the population studied) demonstrating rapid progression of periodontal disease, (2) those (approx. 81%) with moderate disease progression, and (3) those subjects (approx. 11%) who did not demonstrate any signs of disease progression beyond gingivitis.[3] Although bacteria initiate and perpetuate the inflammatory responses in the gingival tissues, the immune-inflammatory responses are the major determinant of susceptibility to disease.[4]

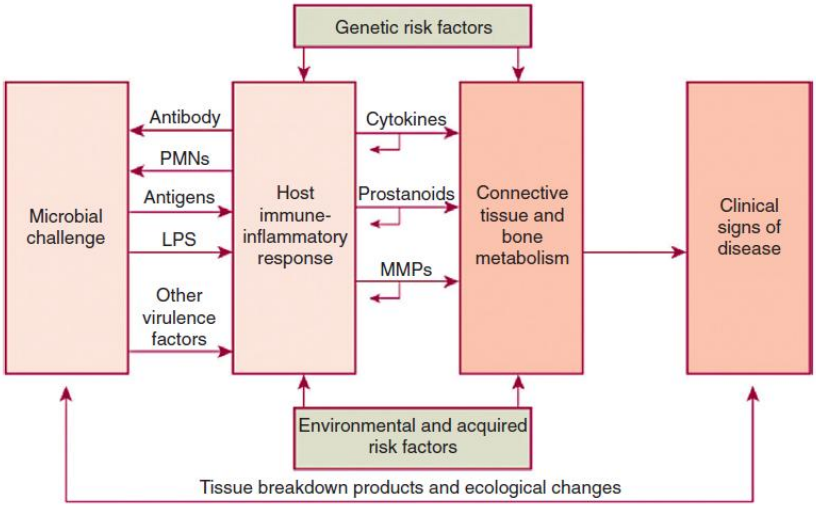


Figure 1: Pathogenesis model- *Modified from Page RC, Kornman KS:* Periodontol 2000 *14:9–11, 1997)* [5]

Many pathological diseases arise as a consequence of inflammation. Among the most common of human diseases, periodontitis was believed to be initiated by specific microorganisms.[6,7] However, it is now widely accepted that, disease initiation and progression can be attributed to multiple factors including; immune-inflammatory response, genetics and various environmental factors (Figure: 1). Periodontal

inflammation, though initiated by bacterial challenge is controlled by the host response to these invaders and are entirely if not partially responsible for the destruction observed in periodontal disease.[8,9] Pathogenic bacteria trigger the inflammatory response in the periodontium. This response, that is protective by intent, results in vascular dilation, increased blood flow, increase in capillary permeability and leukocyte migration into the tissues.[10]

As part of the normal immune mechanism, the monocytes and macrophages are recruited to clear the cellular debris, formed as a result of polymorphonuclear leukocyte (PMN) activity. This clearance allow the tissues to return to health without damage. [11,12,13] However periodontal inflammation is often not resolved and becomes chronic when it is unable to overcome the bacterial challenge or as a result of defective or hyperactive immune response. This chronic inflammation causes damage to the host tissues and serves as a source of nutrition for periodontal pathogens. Further, this unresolved inflammation, causes fibrosis, loss of tissue function and structure, pocket formation and bone loss. Hence timely resolution of inflammation is highly essential to shut down and clear the inflammatory cells.[14]

Inflammation can therefore, be considered as having both pro-inflammatory and pro-resolving mechanisms that serve the ultimate purpose of eliminating the pathogens followed by resolution to prevent self-damage.[15] Resolution of inflammation reverses the signs of inflammation, clears the exudates, fibrin and exhausted leukocytes, resulting in restoration of function. Failure of this mechanism is the cause for disease progression.[16]

Regeneration occurs only when tissue injury is minimal and localized. In cases where damage is extensive, healing occurs in the form of repair that is associated with the formation of granulation tissues and fibrous scarring.[17] Hence, acute resolution results in regeneration while, chronic resolution of inflammation leads to repair.

Based on these findings, it is now accepted that, elimination of pathogenic bacteria does not provide a definitive treatment for periodontitis and other treatment modalities must be combined to treat this multifactorial disease.

Studies done by **Jeffcoat *et al.* (1988)** and (**1993)**, **Offenbacher *et al.* (1992)**, **Paquette *et al.* (2000)**, **Williams *et al.* (1981, 1984, 1985 and 2001)** demonstrated that cyclooxygenase inhibitors can be used to modulate the host response and hence halt periodontal disease progression.[18-25]

In mucosal biofilms, a confounding relationship exists between the change in the integrity of the biofilm and the host immune response. It still remains uncertain as to which comes first.[26] It is now established that periodontitis is induced by inflammation which drives the conversion of gingivitis to periodontitis.[8,9,27] Periodontitis is a result of excessive inflammatory response to normal microbiota and this is further exacerbated by the overgrowth of disease associated bacteria.

Tanner *et al.* (2007), found that the periodontal pathogens appeared in the subgingival environment, following and not prior to disease progression and pocket formation.[28]

Hasturk *et al.* (2007), demonstrated that, periodontal pathogens could be eliminated by controlling inflammation.[29]

Studies performed by **Lindhe *et al.* (1980), Listgarten (1988), Mombelli *et al.* (1991), Page and Schroeder (1976), Tanner *et al.* (2007)**, demonstrated that, inflammatory tissue responses in the subgingival biofilm, always occurred prior to the overgrowth of periodontal pathogens.[28,30-33] This claim is further supported by the fact that there is not enough evidence to implicate bacteria in the initial pathogenesis of periodontal disease.[34-36]

Marsh (1994), suggested that inflammatory responses alter the biofilm such that it allows the proliferation of specific bacteria.[37] This concept has been supported by other authors including; **Clarke and Hirsch (1995)** and **Mombelli *et al.* (1991).**[38,39] This theory is also supported by the observation made by **Tanner *et al.* (2007)**, that gingivitis, and not the overgrowth of periodontal pathogens in a prerequisite in the development of periodontitis.[28]

Hajishengallis (2014), demonstrated that periodontal pathogens thrive only due to the presence of inflammation and are believed to be inflammophilic.[40]

Hajishengallis (2011), demonstrated that the presence of few anaerobic bacteria along with commensals induce inflammation within the periodontium which in turn provides nourishment to the periodontal pathogens such as *Porphyromonas gingivalis*, that thrive in inflammatory conditions and significantly contribute towards dysbiosis.[41]

Yost *et al.* (2015), demonstrated that the virulence of bacteria is also influenced by the inflammatory environment. [42,43]

Hence, the prolonged presence of a subgingival biofilm initiates an immune-inflammatory response that is protective by intent but results in bystander damage which

manifests as periodontal disease resulting in edema and erythema of the gingival tissues which in turn make plaque removal difficult due to a deepened sulcus.

Inflammatory infiltration, particularly the neutrophilic component, cause depletion of collagen beneath the epithelium. This in turn results in compensatory epithelial proliferation. Epithelium acts as a physical barrier against invading bacteria. The disruption of this barrier promotes further bacterial invasion and inflammation.[44] The same neutrophils however, also contribute to destruction by means of matrix metalloproteinase-8 and 9 (MMP-8 and 9) that they release in large amounts. Apart from these, neutrophils also release reactive oxygen species along with other enzymes and cytokines that mediate periodontal destruction.[45] Hence, neutrophil hyperactivity has been shown to be a cause for periodontal destruction. However the cause for this hyperactivity remains uncertain.

Fibroblasts are damaged in the process of periodontal destruction, limiting the chances for repair. On the other hand, the epithelium continues to proliferate apically, deepening the pocket, making it ideal for further colonization.[46] Hence a vicious cycle of chronic inflammation and periodontal breakdown is established. Bacteria initiate inflammation which in turn promotes further colonization and periodontal destruction.

As this inflammation advances to the bone, it leads to osteoclast mediated resorption causing further breakdown of the periodontium.

Hence it is safe to presume that inflammation is the key to periodontal disease.

PTXs : TYPES, STRUCTURE AND FUNCTIONS

PTXs derive their name from the Greek words 'Penta' and 'Ragos,' meaning, 'five' and 'berries' respectively.[1] Based on the length of the N- terminal, PTXs can be classified as short and long. Short or classical PTXs include CRP and SAP, while long or fusion PTXs include, PTX-3, neuronal PTX-1 and 2 (NP-1 and 2), neuronal PTX receptor (NPR) and PTX-4.[1,47] PTX-3 is the prototype of the long PTXs (Figure: 2).

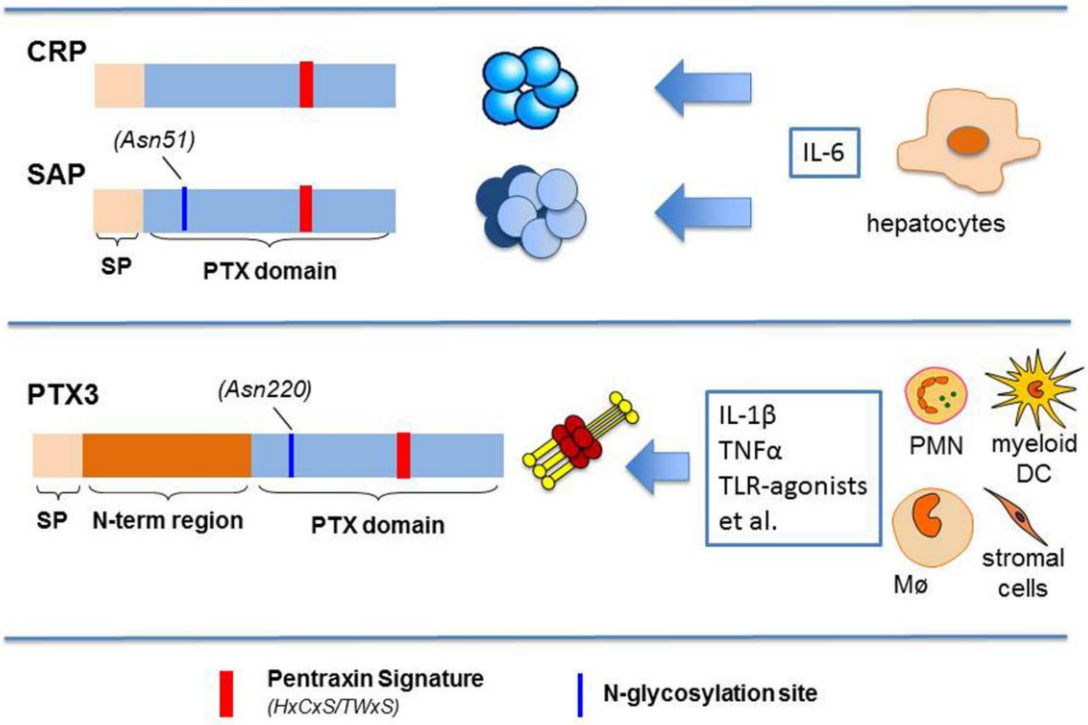


Figure 2: Schematic representation of prototypes of the PTX superfamily[52]

CRP was the first PTX to be discovered in **1929** by **Tillet and Francis[48]** followed by SAP in 1965. While the former was found to be elevated in chronic as well as acute inflammatory conditions, SAP was found to be associated with amyloidosis, spongiform encephalitis and Alzheimer's disease[48] and is not an acute phase inflammatory protein.[49]

PTX-3 was cloned in the 1990's from endothelial cells that were stimulated with interleukin-1 (IL-1) or by treating fibroblasts with tumor necrosis factor- α (TNF-α).[50,51]

THE SHORT PTXs (CRP AND SAP):

Structure:

CRP and SAP have a molecular mass of 115,135 and 127,310 daltons respectively. The short/classical PTXs possess five subunits called protomers that are tightly arranged in planar symmetry.[53] Under an electron microscope, SAP was found to resemble a doughnut shaped ring.[54] Both CRP and SAP circulate as single pentamers in blood.[55] This structure confers the molecules resistance against attack by enzymes along with a high degree of stability.[56] CRP and SAP are structurally and biologically similar with 51% amino acid homology between them.[57] SAP, unlike CRP is glycosylated.[58]

The crystallographic structure of SAP revealed a five-fold symmetry and 4, 6-cyclic pyruvate acetal of β- D galactose and phosphoethanolamine having calcium binding sites.[59] The crystallographic model of CRP was first reported by **Shrive *et al*.** in **1996**.[60] The structure consists of PTX protomers, each made up of two opposing β sheets, forming a β sandwich fold. One face of the protomer contains a hydrophobic pocket, acting as a phosphocholine binding site while the other has an α helix with three turns and contains a C1q as well as a Fc receptor binding site (Figure: 3). A comparison of the properties of the short PTXs is briefly outlined in Table: 1.[53,61,62]

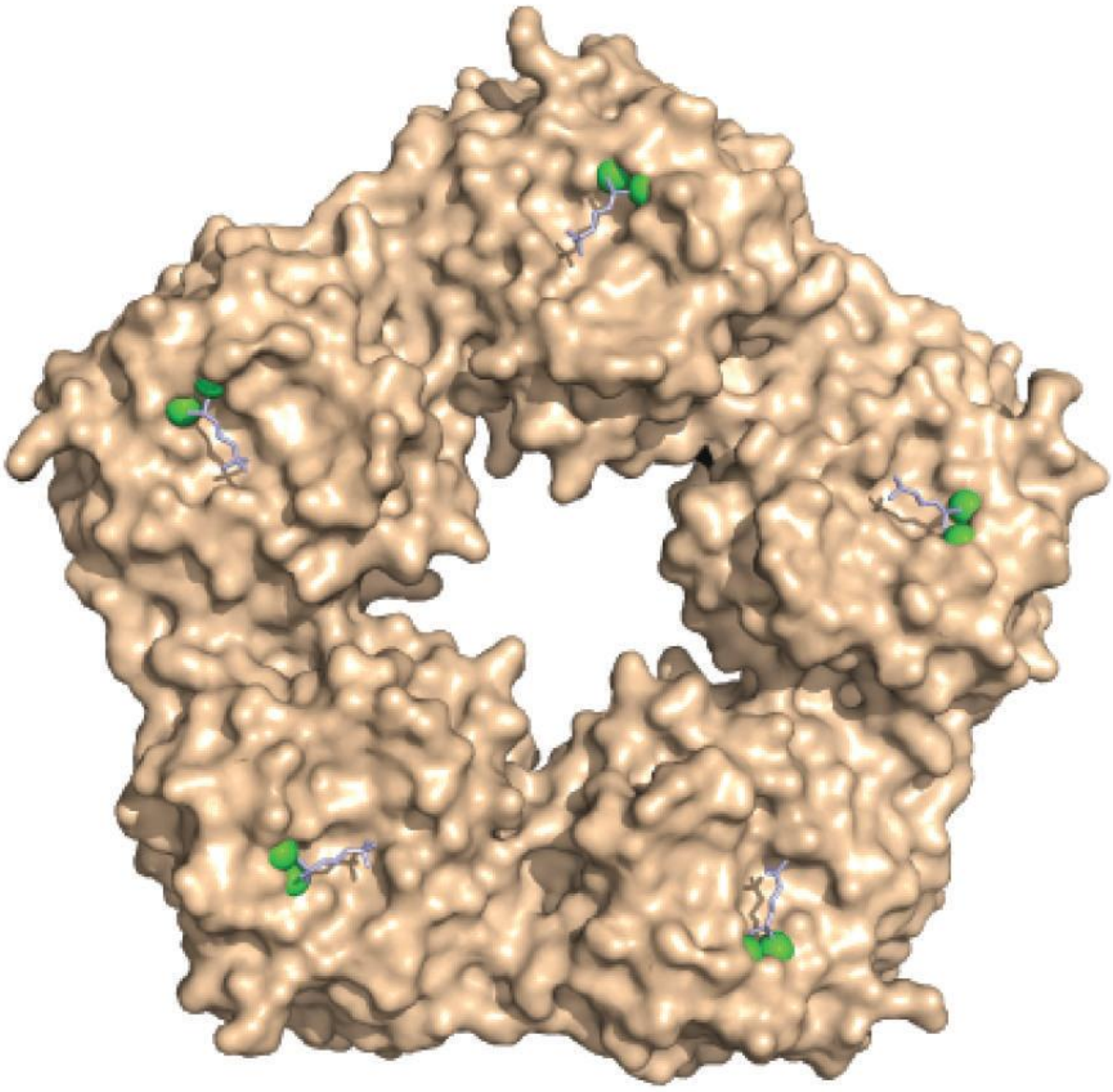


Figure: 3 Surface view of the ligand binding face of CRP. Green indicates the calcium binding sites and blue indicates the phosphocholine binding site.[53]

Table 1: Comparison of the properties of the PTXs: CRP and SAP[53]

	CRP	**SAP**
Fc receptor binding	Yes	Yes
Calcium-dependent ligand binding	Yes	Yes
Complement activation through C1q	Yes	Yes
Ligands	Phosphocholine small nuclear ribonucleoproteins Histones Apoptotic cells Oxidized low density lipoprotein	Phosphoethanolamine Deoxyribonucleotide (DNA), Chromatin Heparin Apoptotic cells Amyloid fibrils
Major synthetic site	Liver	Liver
Inducers	IL-6 (acute phase reactant)	Constitutive
Structure	Cyclic pentamer 115,135Da Each subunit 23,027Da 206 amino acids	Cyclic pentamer 127,310Da Each subunit 25,462Da 204 amino acids
Glycosylation	No	Yes
Chromosomal location	1q23.2	1q23.2

Synthesis:

Both CRP and SAP are synthesized chiefly in the liver. CRP is produced primarily in response to IL-6 and also IL-1β. Among the acute phase proteins in man, SAP and CRP demonstrate the greatest dynamic range. Serum concentrations of CRP may vary from being less than 1µg/mL under normal conditions to 10-500µg/mL during acute phase reactions. CRP levels peak at 48 hours following initiation of an acute phase response.[53,64] Levels of CRP plummet just as rapidly following resolution of this response unlike erythrocyte sedimentation rate levels that remain elevated for a much longer

duration. SAP has been reported to be present in concentrations of 33µg/mL and 43µg/mL in healthy females and males respectively and is not an acute phase protein in humans.[53,65]

Functions: The major functions of the classical PTXs have been briefed in Figure: 4

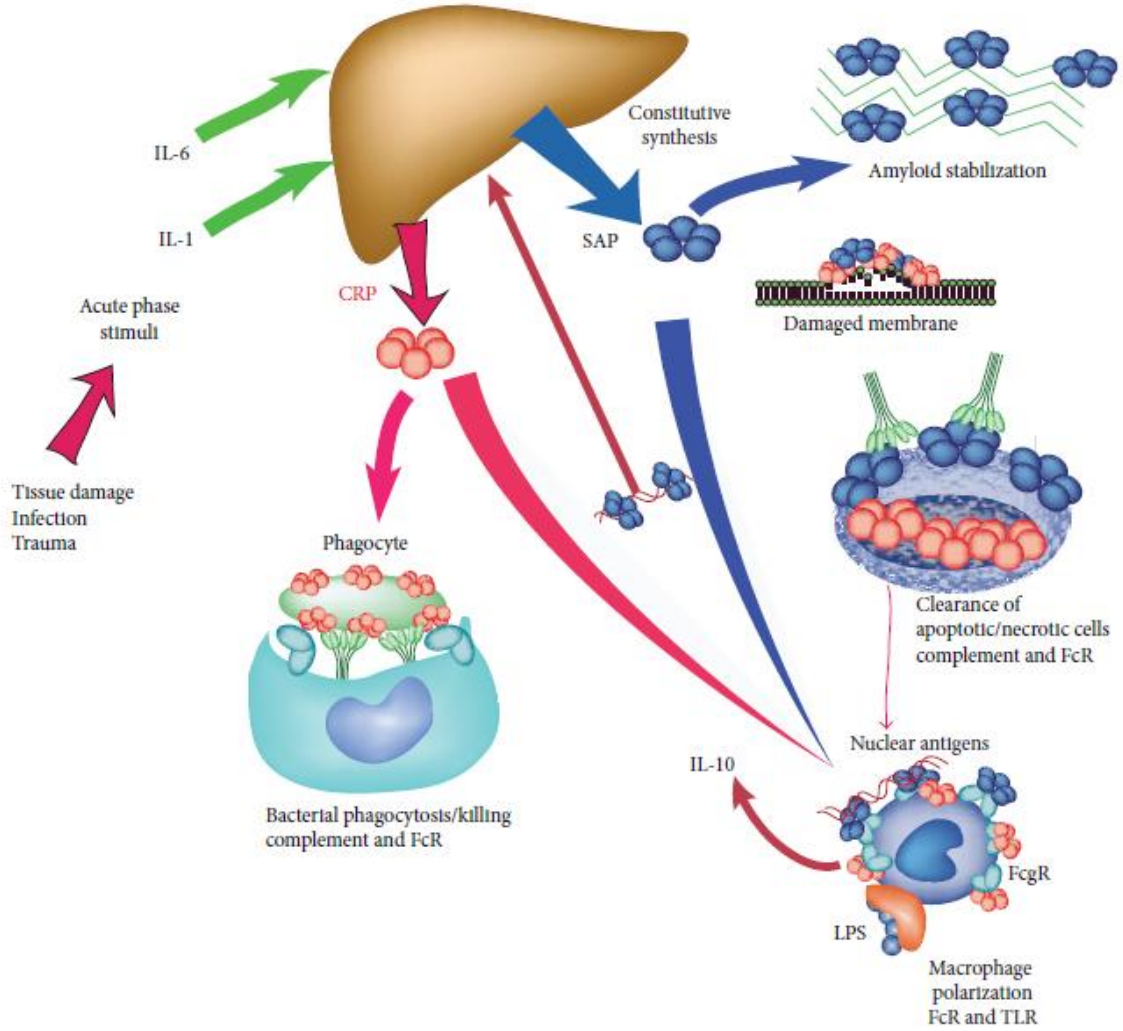


Figure: 4 Overview of the major activities of the short PTXs. Both CRP and SAP contribute to host defense as direct opsonins and through complement activation. Both bind to ligands exposed during cell death and tissue damage leading to opsonization and removal. In addition to these activities many studies support a role for PTXs in regulating the inflammatory response to immune complexes and TLR agonists. This regulation is initiated by PTX interactions with FcγR and mediated by polarized macrophages.[53]

- **Binding to ligands:**

CRP derives its name from the C-polysaccharide present in the cell wall of *S. pneumoniae* with which it interacts.[53,66] CRP plays a major role in the innate immune response by processing the dead and damaged cells.[53,67,68] These damaged cells can stimulate the immune response by the release of various antigens, some of which are targeted by autoantibodies in diseases of the connective tissue such as Systemic Lupus Erythematosus (SLE) wherein, antibodies target host DNA and ribonucleoprotein complexes.[53,69] Clearance and processing of these antigens is affected as CRP and SAP bind to them. While CRP binds to snRNPs.[53,70] SAP binds to DNA and chromatin.[53,71,72] Amyloid fibrils contain SAP and due to its ability to bind to amyloid, SAP helps in localization of deposits in those suffering from amyloidosis.[53,73]

- **Action on complement:**

CRP has the ability to bind to C1q and activate the classical pathway.[53,74,75] This action of CRP renders it invaluable due to its effects on immune and inflammatory responses.[53,76]

- **Receptor binding:**

CRP further influences the innate immune response by binding to Fcγ receptors.[53]

- **Protection from infection:**

CRP has an opsonizing action on *S. pneumoniae* and *E. coli* as demonstrated by **Kindmark** in **1969, 1971** and **1972**.[53,77-79] Like immunoglobulin A, CRP may provide barrier function and by activating complement, it may protect against respiratory tract pathogens. SAP preferentially binds to phosphoethanolamine but has also been demonstrated to bind to *S. pneumoniae*, resulting in complement activation and

phagocytosis. Therefore, CRP and SAP both protect against *S. pneumoniae*, which is a common and fatal infection that affects the old and the young alike. Shiga toxin 2, produced by *E. coli* strains causing hemolytic uremic syndrome and hemorrhagic colitis is neutralized by SAP.[53,80] SAP has also been reported to inhibit infection caused by influenza virus.[53,81,82] It has also been associated with invasive forms of *C. albicans* and with the resulting amyloid deposits formed in the gut.[53,84]

- **Role in Autoimmune disease:**

Deposits of CRP found in the nuclei of cells in the synovium of patients suffering from rheumatoid arthritis[53,85] as well as in neutrophils found in vasculitis[53,86] and allergic encephalomyelitis[53,87] suggest that it may have a role in autoimmune diseases. SAP binds to chromatin and DNA in SLE suggesting a role in the pathogenesis of this disease. It has been suggested that SAP may have a role in the clearance of autoantigens in SLE.[53,88]

- **Action on monocytes and macrophages:**

Classical PTXs reportedly bind to monocytes and neutrophils in a preferential manner and aid in phagocytosis by opsonization of targets via FCγR and FCγRI receptors[53,89-92] and by activating complement.[53,93]

- **Action on dendritic cells:**

CRP may also enhance antigen presentation and immunization against pathogenic microrganisms and enhance their uptake by dendritic cells and macrophages. SAP reportedly binds to DNA, a Toll like receptor 9 (TLR9) agonist and blocks immune responses against DNA vaccines.[53,94]

- **Neutrophil activation, chemotaxis and phagocytosis:**

CRP can opsonize both gram positive and negative organisms, assisting neutrophil activity.[53,78,95] It has also been reported that CRP inhibits neutrophil chemotaxis.[53,96,97]

- **Effects on the vascular endothelium:**

The role of CRP in cardiovascular disease has been widely studied. CRP has been suggested to have a direct inflammatory action on the endothelial cells. It was found that CRP in serum could increase the levels of adhesive molecules by ten-fold though the mechanism remains unclear. As a result CRP can cause vascular injury and cardiovascular disease. The effect of CRP on endothelial nitric oxide synthase expression has been debated. It was suggested that decreased nitric oxide production by CRP action leads to attraction of monocytes and vascular endothelial cells. This theory was opposed by others who suggested that CRP results in increased production of nitric oxide instead. CRP may also cause damage to the vascular endothelium by stabilization of GADD153 messenger ribonucleic acid, resulting in apoptosis of endothelial cells.[53,98]

- **Clinical uses of CRP:**

CRP levels can be used to indicate infection or inflammation. Increased levels of CRP i.e., 10-500µg/mL indicates an acute phase response and is routinely used to measure disease activity in rheumatoid arthritis and other diseases. CRP levels are also used to indicate risk for cardiovascular events and is considered to be as reliable as cholesterol levels that serve as a marker for the same.[53,99]

- **CRP in sepsis and shock:**

Levels of CRP are highly elevated in sepsis. CRP activates complement[53,100] and reduces the levels of C5aR present on neutrophils.[53,101] It has also been suggested that CRP could result in shedding of IL-6 receptors on neutrophils.[53,102] Mice subjected to inflammation by injecting LPS, Platelet activating factor or TNF-α and IL-1β were protected from endotoxic shock due to elevated CRP levels.[53,103] In humans elevated CRP levels were associated with decreased levels of TNF-α and IL-6 when subjected to LPS challenge.[53,104] CRP may activate monocytes to release cytokines IL-10 and interleukin 1 receptor antagonist (IL-1RA), which have anti-inflammatory action. The anti-inflammatory action of CRP may help in preventing shock following trauma.[53,105]

- **SAP in disease:**

SAP is a protein that is constitutively expressed in humans. It is elevated in a number of conditions associated with increased amyloid deposition that affect organ function by parenchymal infiltration. It has also been suggested that SAP protects amyloid fibrils from degrading enzymes.[53,106] In **2003, Pilling *et al*.** reported that SAP could delay wound healing by preventing fibrocyte generation, it does so by binding to FcγR, and specifically to FcγR1.[53,107,108] **Haudek *et al*. (2008)** performed in vitro studies on a mouse model with fibrotic cardiomyopathy and demonstrated that SAP down regulates the conversion of monocytes to fibroblasts.[53,109]

LONG PTXs- PTX3:

Structure:

PTX-3 in humans is a glycoprotein made up of 381 amino acids[110-113] that contain N- and C- terminal domains.[110-112] The N- terminal, composed of 178 amino acids is a

unique feature of the long PTXs while the C- terminal that is composed of 203 amino acids, is common to all PTXs with a 57% similarity between CRP and SAP.[110-112]

The N- terminal portion is composed of four α helices namely, αA, αB, αC and αD that are connected and stabilized by short loops[110-112] (Figure: 5). Three cysteine residues present in inter-chain disulphide bridges that form PTX-3 multimers also have a similar function.[110,112,114] The primary sequence residues (85-91) of the αB helix contains a repeat motif made up of 7 amino acids (abcdefg) where 'a' and 'd' positions are occupied by hydrophobic amino acids while 'e' and 'g' represent positively or negatively charged amino acids.[110,112]

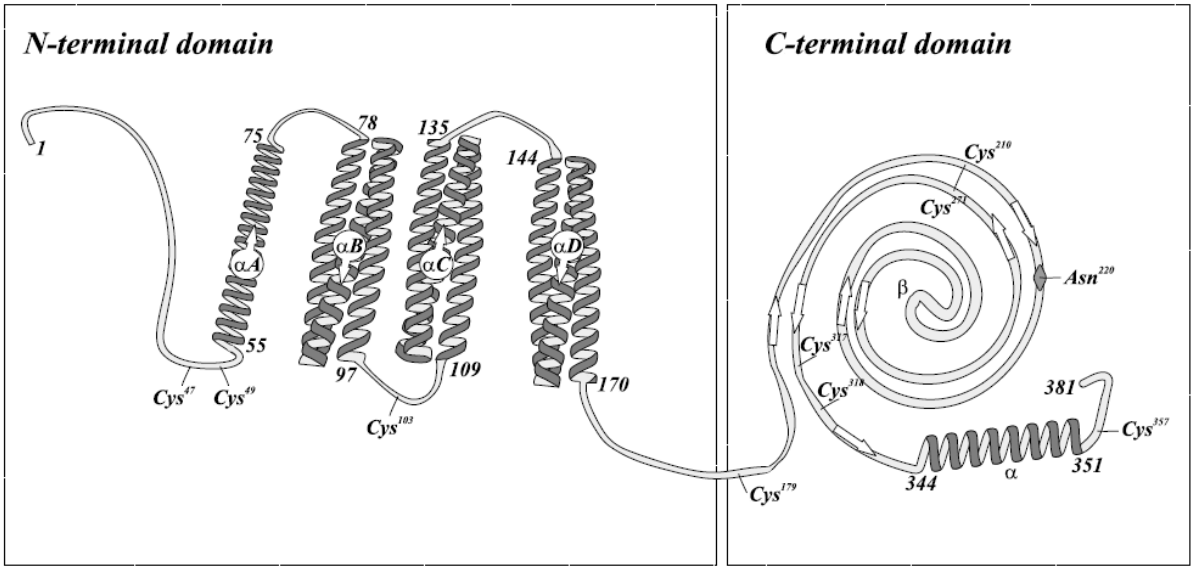


Figure 5: Proposed secondary structure of PTX-3: a structural model. The N-terminal domain consists of four α-helices (αA, αB, αC and αD) connected by short loops. The C-terminal domain is composed of two anti-parallel β-sheet polypeptides organized in a typical β-jelly roll topology and a single α-helix. Asn220 is the unique glycosylation site of PTX-3 moiety.[110]

The C- terminal domain is made up of two anti-parallel β sheets made up of polypeptides that are held together at positions 210 and 271 by covalent bonds between the cysteine residues. The protein surface contains an α helix extending from

amino acid residues 344-351.[110,112] The C- terminal also contains the PTX signature (His-x-Cys-x-Ser/Thr-Trp-x-Ser) where 'x' stands for any amino acid. [110,112,113]

PTX-3 occurs as multimers composed of 8 identical protomers that are connected by disulphide bonds[110,114] each having a molecular weight of 440 kDa.[110,112] PTX-3 has an asymmetric structure and is made up of a dimer of dimers linked by non-covalent bonds and a tetramer linked by disulphide bonds.[110,114] The two tetramers are linked by inter chain bridges at Cys 317 and Cys 318 at the C- terminal forming an octamer. [110,112,113]

The N- terminal region of PTX-3 determines the multimerization of the protein and is responsible for its quarternary structure. The function of the PTX-3 molecule and the number of ligand binding sites are determined by its oligomerization. It has been suggested that PTX-3 has two binding sites for Fibroblast Growth Factor 2 (FGF2) (one in each tetramer). While the inter-α- inhibitor binds to the N- terminal, the C1q binds to the C- terminal domain. Both C- and N- terminal domains interact with Factor H (FH) of the complement.[110,114]

A full size PTX-3 molecule with N- glycosylation site at Asn220 in the C-terminal domain is essential for the process of ligand binding.[110,115] The glycosylation of PTX-3 is essential for its proper conformation, multimer formationand for its interaction with C1q of the complement pathway[110,116] apart from its role in FH interaction and molecule stabilization.[110,115] The general features of CRP and PTX-3 have been compared in Table: 2.

Table 2: General features of CRP and PTX-3[117]		
	CRP- short PTX family	**PTX-3- long PTX family**
Gene location	1q23.2	3q25
Exons	2	3
Glycosylation	No	N- glycosylation site at Asn220
Multimeric formation	Pentamer with non-covalent interactions	Octamer with intra-molecule disulfide bonds
Binding sequences in protomer region	HFN1α, C/EBPβ/δ, STAT, p50 and c-Rel	Pu-1, AP1, NFκB, SP1 and NF-IL6
Major stimulus	IL- 6	TLR agonists, IL-1 and TNF
Producers	Liver (hepatocytes)	monocytes, macrophage, PMN, endothelial cells, dendritic cells, fibroblasts, epithelial cells

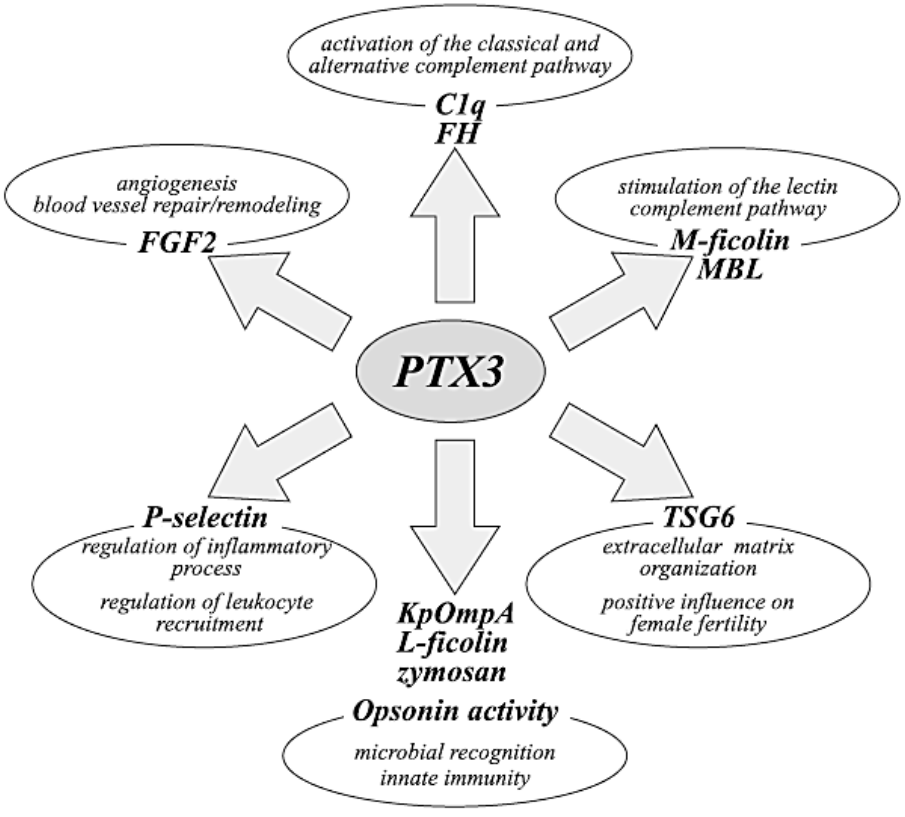


Figure 6: Proposed ligands of PTX-3 and their functions in vivo.[110]

PTX-3 ligands:

PTX-3 may occur in the form of an ECM associated molecule or freely in circulation. The unique N-terminal domain of PTX-3 makes it distinct from the classical PTXs.[110,112] PTX-3 effects its function by binding to various ligands such as: FH, C1q, ficolin-1, mannose- binding lectin (MBL), FGF2, TNF-α induced protein 6 (TSG6) and Outer Membrane Protein A (OmPA) of *Klebsiella pneumoniae*[110,111,118] (Figure: 6). It can also interact with apoptotic cells.[110,119,120]

Functions:

In healthy individuals, the serum and tissue PTX-3 levels are very low. It rises rapidly when exposed to various inflammatory stimuli from below 2ng/mL in health to 200-800ng/mL in inflammation.[110,112,121,122] Serum PTX-3 levels have been reported to be elevated in conditions such as; acute phase of myocardial infarction, cardiovascular disease, unstable coronary artery disease, small vessel inflammation seen in Churg-Strauss syndrome, microscopic polyangitis, systemic inflammatory response syndrome, Wegener's granulomatosis, autoimmune diseases, systemic scleroderma, psoriasis, rheumatoid arthritis, various infectious diseases such as tuberculosis and dengue fever, sepstic shock and sepsis.[110,112,121-124] Endothelial dysfunction in pre- eclampsia also leads to elevation of PTX-3 levels.[110,112] Levels of PTX-3 may also serve as an indicator of disease activity and severity. Table: 3 outlines the various actions of PTX-3 in inflammation and in immunity.[110-112,121]

Table 3: Mechanisms of PTX-3 in inflammation and innate immunity[117]

Effects exerted by PTX-3	**Suggested and/or reported mechanisms by PTX-3**
Protection against infections	- Facilitates phagocytosis of pathogens through complement, complement receptor and Fcγ receptor.[125,126] - Enhances viral clearance, suppresses neutrophil infiltration and inflammatory mediators in murine hepatitis virus-1 induced lung injury.[127] - Exerts reversible effects depending on bacteria burden (*K. pneumoniae*), suppressing neutrophil infiltration and increasing TNF-α level in higher inoculation, while facilitating neutrophil infiltration and without affecting TNF-α level in lower inoculation.[128]
Protection against acute myocardial infarction	- Reduces no-reflow area, IL-6 level, neutrophil infiltration and C3 deposition.[129]
Protection after ischemic stroke	- Reduces blood–brain barrier damage, and participates in the resolution of edema and glial scar formation.[130]
Protection against lung injury	- Reduces neutrophil infiltration, cell death and fibrin deposition in LPS-induced acute lung injury.[131,132]
Protection against LPS damage	- Controls IL-10 production, and enhances nitric oxide production from macrophages in a model of endotoxemia.[133]
Protection against acute kidney injury	- Prevents leukocyte recruitment and abrogates acute renal failure.[134]
Detrimental effects	- Facilitates neutrophil infiltration and proinflammatory cytokine levels (TNF-α, IL-1β, chemokine ligand 1 and 2) in a model of intestinal ischemia and reperfusion.[135,156] - Increases IL-1β, chemokine ligand 2 and chemokine ligand 1 mRNA level in ventilation-induced lung injury.[137]

PTX-3 and apoptotic cells:

PTX-3 binds to cellular debris and apoptotic cells, preventing their presentation and capture by dendritic cells. As a result the activation of the immune system and destruction of the host tissue is limited.[110,111] At the same time, PTX-3 helps in presenting antigens to cytotoxic lymphocytes and induces the synthesis of pro- inflammatory cytokines, thereby guarding against microbial challenge.[110,111,138] PTX-3 plays a dual role by regulating the clonal expression of autoreactive lymphocytes on one hand and protects against bacterial and fungal invasion by means of the innate inflammatory response on the other hand. In this way, PTX-3 protects the host against injury from self as well as against invading pathogens.[110,111]

PTX-3 aids the immune mechanism by means of pathogen recognition and opsonization, complement activation, release of cytokines, maturation of immune cells and activation of glycosylation dependent inflammatory responses.[110,139,142] It also prevents cross presentation of antigens, thereby increasing tolerance to host antigens.[110, 139] Single nucleotide polymorphism in PTX-3 may also be responsible for vascular complications that result from autoimmune diseases.[110,139,142]

PTX-3 as a marker for cardiovascular damage:

PTX-3 can serve as a marker for atherosclerosis, vascular damage, angiogenesis and restenosis.[110,112] PTX-3 has been found to be deposited in ischemic/necrotic heart tissues, implicating it in the injury and repair of the myocardium.[110,112,143] PTX-3 localized in neutrophils during the early stages of acute myocardial infarction suggest that this protein maybe pre- stored in neutrophils, ready for rapid release. In more advanced cases, PTX-3 is produced by the endothelium and macrophages.[110,143] Plasma levels of

PTX-3 increase in acute and chronic coronary syndromes and following stenting.[110,143-145] PTX-3 levels peak 7 hours following the onset of symptoms of myocardial infarction.[110-112,143,144,146] PTX-3 can also serve as an indicator of mortality in patients with acute myocardial infarction following coronary artery bypass graft restenosis.[110,144]

PTX-3 and angiogenesis:

PTX-3 can inhibit the action of FGF2 by binding to it. FGF2 is responsible for migration and proliferation of fibroblasts during wound healing and of vascular smooth muscle cells during restenosis and atherosclerosis.[110,123] PTX-3/FGF2 complexes can also inhibit tumor growth by inhibiting angiogenesis.[110,112]

PTX-3 and the development of atherosclerotic lesions:

PTX-3 has been found in endothelial cells, subendothelial smooth muscle cells, foam cells macrophages and extracellular matrix in atherosclerotic plaques.[110,112] Synthesis of PTX-3 by endothelial cells is stimulated by TNF-α, IL- 1β and bacterial LPS.[110,111] In atherosclerotic lesions however, macrophages and vascular smooth muscle cells that are stimulated by atherogenic lipoproteins (degraded forms of low density lipoprotein) result in PTX-3 synthesis.[110-112] These lipoproteins achieve the same effect by stimulating the release of IL-1 and IL-6 which in turn promotes PTX-3 synthesis.[110,112] PTX-3 can impair the dendritic cell dependent clearance of foam cells by opsonizing apoptotic cells. PTX-3 can also have thrombogenic and prothombotic effects as it stimulates the release of tissue factor by endothelial cells and activated monocytes.[110-112,123,118,146] The FGF2/FGFR system causes proliferation and migration of vascular smooth muscle cells thereby contributing to restenosis due to vessel damage. By binding

to FGF2, PTX-3 prevents restenosis by suppressing chemotaxis and mitosis of the vascular smooth muscle cells.[110,112]

PTX-3 and female fertility:

PTX-3 plays a role in the organization of the ECM and may effect female fertility. PTX-3 maintains the normal structure of cumulus oophorous. Deficiency of PTX-3 leads to uniform expansion of cells in the ECM with an abnormal organization of cumulus oophorous. As a result, despite the normal appearance of oocytes, female fertility is disturbed.[110,119]

PTX-3 levels are increased in pregnancy[147,148] and higher in those complicated by pre- eclampsia. [147,149,150] The plasma and vaginal levels of PTX-3 are elevated in pre-term delivery and in placental vasculopathy.[147,151]

The negative effects of PTX-3:

Despite its many protective functions, PTX-3 has certain negative effects:

- Faster lethality in higher *Klebsiella pneumoniae* inocula
- Worsens tissue injury in:
    - Post- ischemic renal injury
    - Intestinal ischemia and reperfusion
    - Ventilation- induced lung injury (Figure: 7)

Hence the function of PTX-3 differs, depending upon the condition/disease.

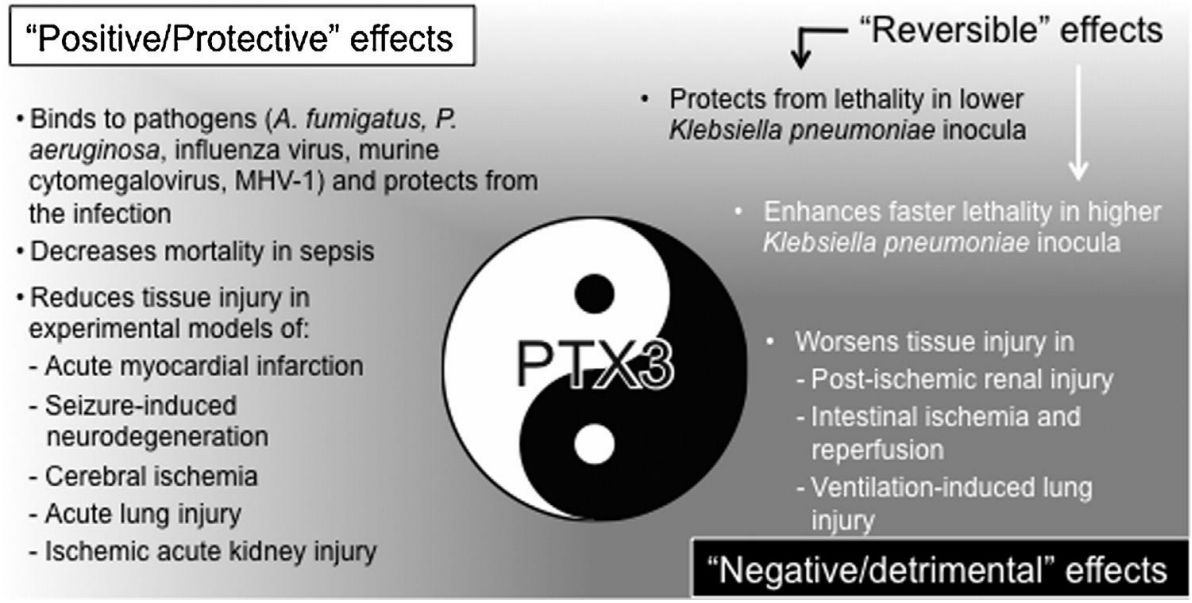


Figure 7: The "yin-yang" effects of PTX-3- The protective effects of PTX-3 are shown in black text and the detrimental effects of PTX-3 are shown in white text. The upper half of the panel assigns infectious conditions and the lower half assigns sterile inflammatory conditions.[117]

Neuronal PTXs and PTX-4:

PTX-4, another member of the PTX family is composed of 470 amino acids. It is 30% identical to the other long PTXs. It is not induced by IL-1 and LPS and is expressed by endothelial cells, monocytes, lymphocytes, leukocytes, natural killer cells and neutrophils.[1,152]

The neuronal PTXs include NP-I, NP-2 and NPR. They are 50% identical to one another and are 20-25% homologous to the short PTXs.[1]

The structure of NP-1 is distinct and consists of five homologous subunits, arranged in radial symmetry to form a pentamer. The expression of this glycoprotein is restricted to the nervous system and is involved in synaptic remodeling and synaptogenesis. Decrease in potassium levels reportedly increases the levels of NP-1 in granule neurons of the cerebellum. Hence hypokalemia associated with xerostomia or injection of local anesthesia, could potentially result in increased levels of NP-1.[1,153,154]

NP-2, also referred to as neuronal activity- regulated protein (Narp), facilitates the outgrowth of neurons and is believed to help in the clearance of degraded synaptic material during formation and remodeling of the synapse as well as, help in aggregation of receptors for neurotransmission at synapses.[1]

NPR is expressed primarily in the central nervous system. Its levels in the cerebrospinal fluid could potentially serve as a biomarker for neurodegenerative diseases such as Alzheimer's disease.[1,155]

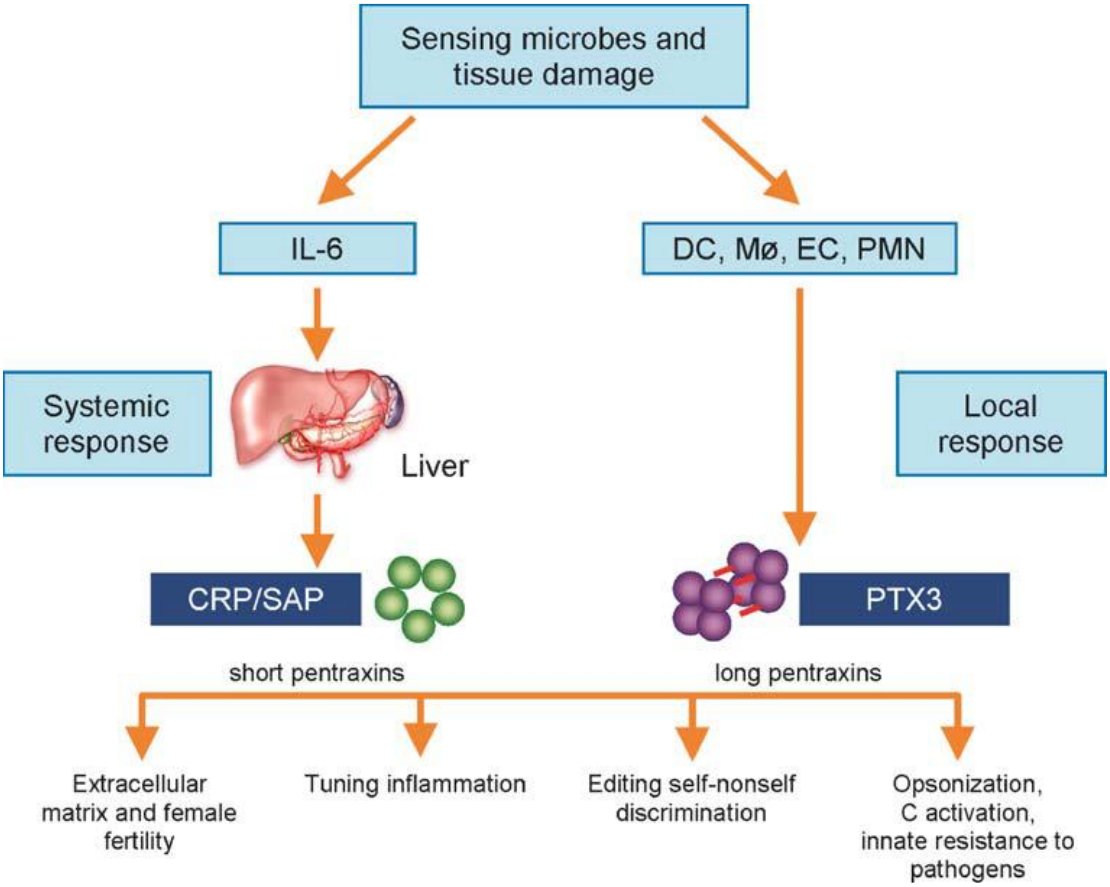


Figure 8: PTXs in innate immunity-an overview-Liver derived short PTXs (e.g., CRP and SAP) and tissue expressed long PTXs (e.g., PTX-3) are produced in response to microbial sensing and inflammatory cytokines and are likely to fulfill complementary functions in innate resistance to pathogens, tuning of inflammation, editing self–non-self discrimination and participating in ECM architecture and female fertility.[147]

Concluding Perspective:

CRP was the first identified innate immune molecule that is capable of recognizing microbial moieties.[147,156] Despite its use as a diagnostic tool, the function of CRP has not been clearly defined. On the other hand, targeting the PTX-3 gene has defined the role of the entire PTX family in innate immunity, inflammation, matrix deposition as well as in female fertility. Figure: 8 gives a brief overview of the role of PTXs in the innate immune response.[147,156]

PENTRAXINS IN PERIODONTAL DISEASE

The relationship between periodontitis and CRP has been widely studied. The systemic impact of periodontal inflammation has been of great interest and various investigations have been carried out linking periodontitis with systemic diseases. Similar literature as regards to PTX-3 and other pentraxins are limited. The value of PTX-3 as a biomarker lies in its local and immediate elevation which could serve as a site specific marker of disease activity.

PTX-3 in periodontal disease:

Pradeep *et al*. (2011)[157] estimated the levels of PTX-3 in the GCF and plasma of 40 subjects (20 females and 20 males; age range: 23 to 50 years) by means of enzyme linked immonosorbent assay (ELISA). Based on the clinical periodontal parameters [gingival index (GI), PD and CAL], the subjects were categorized as being healthy [(H) (n = 10)], having gingivitis (n = 15) and periodontitis (n = 15). The mean GCF concentrations of PTX-3 were 1.96±0.91ng/mL, 2.83±1.23ng/mL and 3.38±1.45ng/mL in H, gingivitis and periodontitis groups respectively which demonstrated a positive correlation with disease progression. The plasma levels of PTX-3 demonstrated a similar trend with values of 1.60±1.12ng/mL, 2.49±1.20ng/mL and 3.07±0.72ng/mL respectively in H, gingivitis and periodontitis subjects. The levels of PTX-3 demonstrated a positive correlation with the clinical parameters. The difference in the levels of PTX-3 among controls and periodontitis affected subjects was statistically significant ($p < 0.01$). The differences in plasma levels of PTX-3 was not significant. It was concluded that GCF values of PTX-3 can serve as a marker of inflammation in periodontal disease.

Fujita *et al*. (2012)[158] performed a study to determine the GCF levels of IL-1β, IL-10, IL-8, IL-6, TNF-α and PTX-3 from diseased and healthy sites in 50 patients with chronic periodontitis (CP). GCF samples were collected using paper strips from one periodontally diseased and one periodontally healthy site in every subject. The levels of PTX-3 level was measured using ELISA while those of IL-1β, IL-10, IL-8, IL-6 and TNF-α were determined using a multiplexed bead immunoassay. The clinical periodontal parameters were significantly higher at diseased sites ($p < 0.01$) as compared to healthy sites. The mean levels of PTX-3 in the diseased and healthy sites were 0.64±0.39ng/mL and 0.06±0.10ng/mL respectively. IL-1β demonstrated levels of 4.93±5.27pg/mL and 0.20±0.31pg/mL, IL-6 displayed levels of 0.51±0.89pg/mL and 0.11±0.19pg/mL, IL-8 demonstrated levels of 50.50±19.18pg/mL and 25.06±15.72pg/mL, IL-10 demonstrated levels of 0.67±1.17pg/mL and 0.27±0.54pg/mL while TNF-α displayed levels of 0.33±0.33pg/mL and 0.17±0.31pg/mL in the diseased and the healthy sites respectively. The mean levels of the biochemical markers namely, PTX-3, IL-1β, IL-6, IL-8, IL-10 and TNF-α were higher and statistically significant in the diseased when compared to the healthy sites ($p < 0.01$). A strong positive correlation was found between the clinical parameters when correlated with PTX-3 as well as with IL-1β.

Keles *et al*. (2012)[159] tested the hypothesis that levels of PTX-3 in serum and gingival tissues increases in a model of experimental periodontitis by evaluating the levels of the same at 10 and 40 days following the induction of experimental periodontitis in rats. A total of 30 rats were equally divided into 3 groups namely; H group ($n = 10$), ligature-induced experimental periodontitis groups- 10 day period ($n = 10$) and 40-day period ($n = 10$). The rats were sacrificed at the end of the study period following which

histomorphometric and radiological analyses were performed on the mandibles. Concentrations of PTX-3 in gingival tissue and serum samples were estimated using ELISA while the fibrinogen levels in plasma were measured by the nephelometric method. Significant periodontal inflammation and resorption of alveolar bone were evident in periodontitis groups. PTX-3 levels in the gingival tissues of the periodontitis induced animals when studied at day 10 (1.6±0.4ng/mL) were statistically higher than those in the 40 day group (1.2±0.2ng/mL) and controls (1.1±0.2ng/mL) ($p < 0.01$). The serum levels of PTX-3 did not demonstrate any significant difference between the experimental and control groups ($p > 0.05$). The levels of plasma fibrinogen were significantly higher in the experimental periodontitis groups ($p < 0.001$). It was concluded that PTX-3 seems to be associated with destruction of tissues in early periods of periodontal inflammation.

Surlin *et al*. (2012)[160] measured the GCF levels of PTX-3 in patients during the first 2 weeks of orthodontic treatment to assess if the values demonstrated any variation and if the changes observed were the result of an inflammatory state. A total of 39 subjects were included of which 16 were young orthodontic patients and 13 were adults requiring distalization of the maxillary canine (the contralateral canine acted as the control). Paper strips were used to collect GCF at different intervals (1 hr before treatment, 4, 8, 24 and 72 hours, 1 week and 2 weeks after initiation of orthodontic therapy) and the PTX-3 levels were determined by means of ELISA. The PTX-3 levels at 1 hour, 4, 8, 24, 72 hours, 1 week and 2 weeks were found to be 1.05±0.67ng/mL, 1.37±0.75ng/mL, 2.29±0.58ng/mL, 2.69±0.64ng/mL, 2.38ng/mL±0.78ng/mL, 1.34±0.65ng/mL and 1.06±0.77ng/mL respectively in young patients and

1.053±0.64ng/mL, 1.28±0.54ng/mL, 1.47±0.50ng/mL, 2.146±0.65ng/mL, 1.67±0.91ng/mL, 1.043±0.60ng/mL and 1.046±0.60ng/mL respectively in adult patients. The levels of PTX-3 were found to be the maximum at 24 hours followed by a decrease. The return of PTX-3 levels to baseline levels occurred within 2 weeks for young and in 1 week for the adult patients. These results indicate that PTX-3 has a role in aseptic inflammation associated with orthodontic force.

Elgendy *et al*. (2013)[161] evaluated the effectiveness of tea tree oil (TTO) when used as an adjunct to non- surgical periodontal therapy in subjects with CP. The levels of PTX-3 in GCF were assayed by means of ELISA following collection using paper strips. The clinical periodontal parameters were recorded at baseline, 1, 3 and 6 months following therapy. Subjects (n = 40) with moderate to severe CP were divided into 2 groups. One group received scaling and root planing (SRP) only, whereas the second group received SRP and TTO gel. 5% of TTO gel was filled in the deepest periodontal pocket following SRP after which the area was covered with periodontal pack for 7 days. The clinical parameters demonstrated significant improvement at all time periods in the test when compared to the control group. The PTX-3 levels at baseline, 1, 3 and 6 months were 0.84±0.31ng/mL, 0.25±0.20ng/mL, 0.33±0.25ng/mL and 0.35±0.24ng/mL respectively in the control group and 0.81±0.29ng/mL, 0.10±0.04ng/mL, 0.11±0.04ng/mL and 0.35±0.24ng/mL respectively in the test group. Hence PTX-3 demonstrated a significant reduction in the test group when compared to the controls ($p < 0.01$ at 1 month and $p < 0.001$ at 3 and 6 months). It was concluded that local delivery of TTO gel may augment the results of conventional periodontal therapy in subjects with

CP. Additionally monitoring the GCF levels of PTX-3 may be useful as a marker to evaluate periodontal tissue healing.

Gümüş *et al*. (2014)[162] evaluated the levels of PTX-3 and IL-1β in serum and saliva levels of patients with generalized forms of CP and AgP and in periodontally healthy individuals. 94 subjects [25 CP, 25 AgP and 44 healthy age matched to the 2 test groups] were recruited. Serum and saliva samples were collected and clinical periodontal parameters were recorded. The biochemical parameters namely PTX-3 and IL-1β were estimated by ELISA. Serum and saliva data were similar in CP and AgP groups. The salivary levels of PTX-3 and IL-1β in subjects with CP were 49.2ng/mL (range: 25.6-60.2ng/mL) and 197.3pg/mL (range: 94.2-443.8pg/mL) respectively. The levels of the same biomarkers in subjects with AgP were 48.1ng/mL (range:33.4-65.8ng/mL) and 283pg/mL (range:90.9-604.3pg/mL) respectively. The levels of PTX-3 and IL-1β in saliva of controls age matched to subjects with AgP (n = 22) were, 36.1ng/mL (range: 0.5-46.7ng/mL) and 20.4pg/mL (range: 11.9-90.9pg/mL) respectively. The salivary levels of PTX-3 and IL-1β in controls that were age matched to the subjects with CP (n = 22) and AgP (n = 22) were 37.0ng/mL (range:25.6-48.5ng/ml) and 60.01pg/mL (range:12.7-334.4pg/mL) respectively. The serum levels of PTX-3 in CP and AgP subjects were 1.0ng/mL (range: 0.4-2.8ng/mL) and 0.6ng/mL (range: 0.2-1.7ng/mL) respectively while the levels of IL-1β in CP and AgP subjects were 11.7pg/mL (range: 11.1-15.1pg/mL) and 10.9pg/mL (range: 10.5-12.9pg/mL) respectively in serum. Salivary levels of IL-1β were higher in subjects with AgP than in subjects with CP and this difference was statistically significant ($p < 0.05$). The salivary PTX-3 levels were higher and statistically significant in subjects with AgP when compared to the control group ($p < 0.05$). Salivary levels of

PTX-3 in subjects with CP correlated with bleeding on probing and plaque index (PI) ($p < 0.05$). In the AgP group, the saliva and serum PTX-3 levels correlated with levels of IL-1β ($p < 0.05$). It was concluded that salivary concentrations of PTX-3 may be of diagnostic value in subjects with periodontitis.

Lakshmanan *et al.* (2014)[163] estimated and compared the levels of PTX-3 in gingival tissues of subjects with CP, AgP and in healthy controls. The tissue levels of PTX-3 were also correlated with the clinical periodontal parameters. 50 subjects belonging to age groups of 20 to 55 years were recruited from the patients visiting the Department of Periodontics, Saveetha Dental College and Hospital, Chennai, India and categorized into three groups: 1) Patients with generalized CP (n = 20); 2) Patients with generalized AgP (n = 20); and 3) H subjects (n = 10). Tissue levels of PTX-3 were estimated using ELISA. Patients with generalized AgP demonstrated greater levels of PTX-3 in gingival tissues (8.35±5.08ng/mL) when compared to patients with generalized CP (5.07±3.27ng/mL) and controls (0.25±0.28ng/mL). The clinical periodontal parameters and PTX-3 levels demonstrated a positive correlation in all 3 groups with full mouth probing depth (fPD) being the most significant predictor of PTX-3 levels in subjects with periodontitis. The mean fPD was 3.89±0.76mm, 4.58±1.18mm and 0.54±0.18mm in patients with generalized CP, generalized AgP and in H groups respectively. It was concluded that levels of PTX-3 in tissues can act as a marker of inflammation in periodontal disease.

Nizam *et al.* (2014)[164] evaluated the levels of the IL-1β, IL-6, IL-21, IL-33, and PTX-3 in the saliva of patients with and without obstructive sleep apnea syndrome (OSAS). A total of 52 patients were included in the study. The subjects were categorized

as control (non-OSAS) group (n = 13), subjects suffering from mild/moderate OSAS (n = 17), severe OSAS group (n = 22). Clinical periodontal parameters were measured and saliva samples were collected prior to periodontal treatment. The levels of salivary cytokines were determined by means of ELISA. There was no significant difference in concentrations of IL-6 and IL-33 in the saliva of subjects with either form of OSAS ($p > 0.05$). However the levels of the same in OSAS groups were much higher than in the controls and this difference was statistically significant ($p < 0.05$). Salivary concentrations of IL-1β, IL-21 and PTX-3 did not demonstrate any significant variation among the study groups. Among the clinical parameters, significant correlation was found between clinical attachment level (CAL) and IL-21 ($p < 0.05$). The correlation between PD, CAL and indicators of OSAS severity was found to be highly significant ($p < 0.01$). It was suggested that OSAS may increase the concentrations of salivary IL-6 and IL-33 independent of OSAS severity.

Çalapkorur *et al*. (2016)[165] evaluated the relationship between periodontal disease and peripheral arterial disease (PAD) by assessing the levels of high sensitive C-reactive protein (hs-CRP) and inflammatory cytokines (PTX-3 and IL-1β) from serum and GCF. 60 patients were divided into 2 groups namely non-PAD (control group) and PAD (test group) based on the ankle–brachial index. The test and control groups did not demonstrate any significant differences in terms of the clinical periodontal parameters, age, gender, body mass index (BMI) or smoking history ($p > 0.05$). GCF levels of IL-1 β, hs-CRP and PTX-3 were 1138.6±245pg/mL, 5.96±0.89mg/mL and 2.33±0.44ng/mL respectively in the non-PAD group and 1098.1±242pg/mL, 5.81±1.0mg/mL and 2.68±0.63ng/mL respectively in the PAD group. Serum levels of the same were

3.31±0.4pg/mL, 0.92±0.2mg/mL and 0.62±0.1ng/mL in the non-PAD group and 3.18±0.3pg/mL, 0.92±0.1mg/mL and 0.49±0.1ng/mL in the PAD group respectively. The levels of IL-1β and PTX-3 did not demonstrate significant differences between the test and control groups. Following adjustment for confounding factors (gender, age, hypertension, diabetes and body mass index), logistic regression analysis revealed that, periodontitis increased the odds for having PAD to 5.84 (95% confidence interval: 1.56–21.91). Though there were no significant differences between the study and control groups with respect to clinical periodontal parameters and biochemical analyses, it was demonstrated that periodontitis raised the odds for having PAD.

CRP in periodontal disease:

Salzberg *et al*. (2006)[166] performed a study to determine the levels of CRP in the serum of subjects with AgP and in periodontally healthy individuals. They also attempted to determine the patient characteristics that may explain the differences observed between the groups. Samples were collected from 93 patients diagnosed with generalized AgP, 97 with localized AgP and from 91 H subjects. The serum levels of CRP were determined by means of a high- sensitivity ELISA assay. There was a significant difference observed in the serum levels of CRP between the groups. The concentrations of CRP in controls, localized and generalized AgP were 0.66mg/mL, 1.10mg/mL and 2.05mg/mL respectively. A positive correlation was found between CRP and periodontal parameters suggesting that periodontal infections may contribute to systemic inflammation.

Pejcic *et al*. (2011)[167] measured the serum levels of CRP in 26 patients with severe periodontitis, 24 patients with moderate periodontitis and in 25 periodontally healthy subjects. Patients who had lesser attachment loss and PD of <4 mm were

diagnosed as having moderate periodontitis while those with greater attachment loss and PD of >5 mm were diagnosed as having severe periodontitis. Subjects with healthy gingiva, a sulcus depth of <2 mm and no attachment loss were categorized as healthy controls. Polymerase chain reaction was used to detect the presence of periodontopathogens in subgingival plaque samples. Patients with severe and moderate forms of periodontitis had higher mean CRP levels of 8.25±9.35mol/L and 4.93±3.23mol/L respectively when compared to healthy controls (1.09±1.48mol/L). The presence of periodontopathogens was also associated with poor periodontal status and elevated CRP levels. These results suggest that periodontal infection in otherwise healthy individuals may contribute to systemic inflammatory burden.

Chopra *et al*. (2012)[168] compared the serum levels of CRP and the alveolar bone loss in patients with CP and AgP. 45 subjects participated in the study and were diagnosed as suffering from generalized AgP, generalized CP or acted as non-periodontitis controls. CRP levels in venous blood samples were assessed using turbidimetric immunoassay. Alveolar bone loss was measured on a panoramic radiograph at proximal sites of posterior teeth. Mean levels of CRP were greater in generalized AgP (7.49±2.31 mg/L) and generalized CP (4.88±1.80 mg/L) groups when compared to the controls (0.68±0.23 mg/L) and this difference was statistically significant with p value < 0.001. The mean alveolar bone loss was 36.77% in the generalized AgP group and 31.58% in generalized CP group with the difference being statistically significant (p < 0.01). It was concluded that both forms of periodontitis impact systemic inflammatory response with the degree of impact being determined by the aggressiveness of disease progression.

Chopra *et al*. (2012)[169] compared the levels of CRP in subjects with AgP and CP. 80 subjects with generalized AgP, 80 with generalized CP and 80 without periodontitis were included in the study. All 240 subjects were systemically healthy. CRP levels were analyzed by means of turbidimetric immunoassay following the collection of venous blood. The CRP levels were found to be higher in the subjects with periodontitis (7.49±2.31 mg/L in generalized AgP and 4.88±1.80 mg/L in generalized CP) than in those without periodontitis (0.68±0.23 mg/L). The mean CRP levels in generalized AgP were significantly higher than those in generalized CP. Further, the CRP levels correlated positively with the clinical parameters of periodontal destruction such as PD and CAL in both CP and AgP groups. It was concluded that younger patients with rapid periodontal destruction demonstrated higher levels of CRP which could put them at greater risk for cardiovascular disease and that further research at the community level is required to confirm these findings.

Wohlfeil *et al*. (2012)[170] compared markers of inflammation namely, CRP, serum elastase, IL 6 and 8, LPS binding protein (LBP) and leukocyte counts in the blood samples of patients with CP (n = 31), AgP (n = 29) and in H subjects (n = 30). Levels of CRP, serum elastase, LBP and IL6 were higher in the AgP group than in the control group ($p < 0.05$), however levels of IL-8 and leukocyte counts were similar between these groups. The levels of serum elastase in CP, AgP and in controls were, 17.12±12.35ng/mL, 32.00±14.63ng/mL and 9.99±4.73ng/mL respectively. The CRP levels in these groups were, 0.17±0.23mg/dL, 0.55±0.98mg/dL and 9.99±4.73ng/mL respectively. The levels of both CRP and serum elastase were significantly higher in the

AgP group when compared to the control group suggesting a higher inflammatory burden in patients with AgP.

Eickholz *et al*. (2013)[171] studied a total of 31 CP and 29 AgP subjects. Clinical examinations were made before and 12 weeks after SRP. Patients positive for *Aggregatibacter actinomycetemcomitans* (9 CP and 14 AgP subjects) were prescribed systemic antibiotics. Blood samples were collected before, on day 1, day 6 and 12 weeks following SRP. The biochemical parameters assessed included CRP, Lipopolysaccharide binding protein (LBP), serum elastase, IL-6 and 8 and leukocyte counts. The baseline levels of CRP, LBP and serum elastase were significantly elevated in AgP when compared to CP patients ($p < 0.001$). Levels of serum elastase demonstrated a significant difference between AgP and CP groups ($p < 0.05$) 12 weeks after non- surgical periodontal therapy. Multiple regression analysis revealed that reduction in CRP levels was associated with baseline PD, antibiotic therapy and African origin.

Goyal *et al*. (2014)[172] compared the levels of CRP in the serum of patients with CP and AgP. 75 subjects who were systemically healthy were categorized as belonging to non- periodontitis, generalized CP and AgP groups. CRP levels were estimated by means of ELISA. Mean CRP levels were significantly greater in generalized CP (2.31 ± 0.56 mg/L) and generalized AgP subjects (4.61 ± 0.58mg/L), as compared to non-periodontitis subjects (0.96 ± 0.13mg/L). The levels in the generalized AgP group were higher than that in the generalized CP group. Furthermore, there was a positive correlation between the levels of CRP and the clinical parameters as measured by PD and CAL. The results of this study indicate a positive correlation between periodontal disease severity and CRP levels. It was also suggested that the severity of periodontal disease,

particularly in young subjects could increase the risk for cardiovascular disease by unknown mechanisms as indicated by the elevated CRP levels.

Pradeep *et al*. (2014)[173] evaluated the levels of S100A12 (extracellular newly identified receptor for advanced glycation end products binding protein) and high sensitivity CRP (hs-CRP) in the serum and GCF of CP patients with and without type 2 diabetes mellitus. The study group comprised of 44 subjects of which, 10 served as healthy controls, 17 had CP and 17 were type 2 diabetic with CP. ELISA was used to estimate S100A12 while immunoturbidimetric analysis was employed to estimate levels of hs-CRP in GCF and serum. Levels of S100A12 and hs-CRP were highest in diabetic patients with CP followed by subjects with CP alone and the least in the control group. The serum levels of hs-CRP were 4.47±0.77mg/L, 3.44±0.38mg/L and 2.05±0.78mg/L respectively in these groups, while the GCF levels of the same were, 0.98±0.20mg/L, 0.85±0.17 mg/L and 0.49±0.25 mg/L respectively. It was concluded that, serum and GCF levels of hs-CRP and human S100A12 can be considered as markers of inflammation in diabetes mellitus and in CP.

Podzimek *et al*. (2015)[174] evaluated and compared the levels of CRP in the peripheral blood of patients with CP, AgP, gingivitis and gingival recession. They also compared the values with the clinical periodontal parameters. 158 patients were examined and categorized into groups as; AgP (n = 26), CP (n = 111), gingivitis (n = 13) and gingival recession (n = 8). The CRP levels in these groups were 2.8±2.4 mg/L, 2.2±2 mg/L, 2.1±1.7mg/L, 1.3±0.7mg/L respectively. The results of the study demonstrated that CRP levels increase with increase in the severity of periodontal disease.

de Souza *et al*. (2016)[175] aimed to evaluate the levels of CRP in serum of patients with CP and in periodontally healthy subjects. They also attempted to determine the impact of non- surgical periodontal therapy on the levels of CRP. The subjects included in the study were systemically healthy and did not present with any confounding factors. At baseline, clinical parameters and CRP levels were measured in all subjects. Non-surgical periodontal therapy, comprising of SRP and oral hygiene instruction was administered to subjects in the test group and 60 days following therapy, the clinical periodontal parameters and CRP levels were reassessed. The baseline CRP levels were significantly higher in the test than in in control group (1.98±1.55mg/L vs. 1.26±1.05mg/L; $p < 0.05$). In patients with higher CRP levels at baseline (>3 mg/L) a significant improvement was observed on reassessment at 60 days. The results of this study suggest that levels of CRP are elevated in CP. It was also concluded that non-surgical periodontal therapy causes a decrease in CRP levels in subjects with high baseline levels of the same.

Zhang *et al*. (2016)[176] performed a study on 40 subjects to compare the GCF levels of 5 biomarkers namely; IL-6, IL-10, TNF-α, CRP and alkaline phosphatase by means of ELISA. The study sample included 15 subjects with AgP, 15 suffering from severe CP and 10 H subjects. Baseline GCF samples were collected using paper strips from different sites. Levels of IL-6, TNF-α, CRP and alkaline phosphatase were significantly higher in the CP and AgP groups compared to that in the H group. On the other hand IL-10 levels were lower in the periodontitis groups than in the H group. All 5 biomarkers demonstrated a correlation with the clinical parameters. It was concluded that the biomarkers that were evaluated in this study can be used to estimate disease activity in periodontitis.

Bolla *et al*. (2017)[177] compared the levels of serum CRP in subjects with AgP and CP. Following initial examination, 45 subjects were categorized into 3 groups as; those with clinically healthy periodontium, AgP and CP. The clinical parameters assessed included; PI, CAL and PD. Blood samples were collected and CRP levels were assayed by means of high sensitivity ELISA. The levels of CRP were; 1.02±0.94 mg/L, 4.55±2.88mg/L and 6.07±3.16mg/L in H, AgP and CP subjects with the CP group having greater values but there was no significant difference between the two test groups. It was concluded that the levels of CRP are elevated in periodontal disease.

Chandy *et al*. (2017)[178] evaluated the peripheral blood for levels of plasma fibrinogen and serum CRP in CP (n = 20), AgP (n = 20) and in H subjects (n = 15). Among the 55 subjects included in the study, 28 were female and 27 were male. The periodontal parameters recorded include, simplified oral hygiene index, bleeding index, CAL and PD. The levels of plasma fibrinogen were measured using quantitative immunoturbidimetric assay and serum levels of CRP by high sensitivity ELISA. The levels of plasma fibrinogen as well as serum CRP were higher in periodontitis patients (CP and AgP) when compared to that of H subjects ($p < 0.001$). The mean levels of fibrinogen were higher in the CP group (763.90±247.39µg/mL) when compared to the AgP (616.53±189.49µg/mL) and H group (337.50±158.33µg/mL; $p < 0.001$). CRP levels were similarly higher in patients with CP (3397.00±2511.77ng/mL) than in those with AgP (2692.27±2585.64ng/mL) and in the H group (1112.35±390.38ng/ml; $p < 0.004$). A positive correlation was found between clinical periodontal parameters and levels of CRP and plasma fibrinogen. It was concluded that CRP and fibrinogen levels may help in evaluating the association between cardiovascular and periodontal disease.

STUDY DESIGN:

45 subjects [15 CP, 15 AgP and 15 H subjects] were recruited from the Department of Periodontics, Sri Ramakrishna Dental College and Hospital, Coimbatore. The study protocol was approved by the ethical committee, Sri Ramakrishna Dental College and Hospital, Coimbatore and informed consent was obtained from each patient before enrolling them in the study. The participants were required to fulfill the following criteria:

INCLUSION CRITERIA:

SELECTION OF CONTROLS:

Group 1: Healthy individuals (Control group) (Figures: 16-18)

- Absence of bleeding on probing.
- Probing depth (PD) < 3mm.
- Absence of clinical attachment loss.
- Patients without any systemic disease.

SELECTION OF CASES:

Group 2: Chronic Periodontitis (Test group I) (Figures: 10-12)

- Generalized (or) localized mild, moderate or severe CP patients (According criteria given by Armitage).[179]

Group 3: Aggressive Periodontitis (Test group II) (Figures: 13-15)

- Generalized (or) localized AgP patients – (According to Lang's criteria).[180]

EXCLUSION CRITERIA:

- Patients with history of antibiotic and anti-inflammatory drug intake in the past 3 months.
- Patients who have undergone any form of periodontal therapy in past 6 months.

- Patients with inflammatory systemic diseases that may have an impact on the levels of PTX-3.
- Pregnant/Lactating women.

ARMAMENTARIUM:

Sample collection (Figure: 9)

- Dental mouth mirror
- William's periodontal probe
- Explorer
- Tweezer
- Cotton rolls/gauze
- Universal curettes (4R/4L and 2R/ 2L)
- Head cap
- Surgical mask
- Gloves
- Microcapillary pipettes (10µL, Drummond Scientific Company, Delaware County, Pennsylvania, United States)

Sample storage (Figure: 19 & 20)

- 2mL Polypropylene tubes
- Aluminium foil
- Ultra low temperature freezer (-80ºC)

SAMPLE PROCESSING:

- ELISA kit for detection of PTX-3 (Figures: 21-24)

CLINICAL EXAMINATION:

During examination of the patient, the following clinical parameters were assessed:

Plaque Index:

The full mouth plaque was assessed using the criteria of the Plaque Index system given by **Silness and Löe** as modified by **Löe (1967)**.[181]

Modified Sulcular Bleeding Index:

The modified Sulcular Bleeding Index (mSBI) was assessed using the criteria given by **Mombelli *et al*. (1987)**.[182]

Probing Depth:

The probing depth was assessed on each tooth from the gingival margin to base of the sulcus/pocket using William's periodontal probe at 6 specific surfaces per tooth (distobuccal, midbuccal, mesiobuccal, distolingual, midlingual and mesiolingual surfaces).

Clinical Attachment Level:

The clinical attachment level was recorded from cementoenamel junction to the base of the gingival sulcus/pocket using William's periodontal probe at all the six sites as mentioned for probing depth.

GCF COLLECTION:

GCF samples were obtained using microcapillary pipettes. GCF was collected from sites demonstrating maximum bleeding score as assessed by the mSBI in subjects with CP and AgP and from the most convenient site in H subjects. The chosen site was isolated by means of cotton/gauze. Plaque along with the supragingival calculus was removed with a universal curette to avoid contamination and blocking of the microcapillary pipette. The

sulcular areas were gently air dried and a calibrated (10µL) volumetric microcapillary pipette (Drummond, Broomall, USA)^{TM} was placed at the entrance of the gingival crevice. The GCF samples which were contaminated with blood, saliva or exudate were discarded and fresh samples were collected. Each microcapillary pipette containing GCF was wrapped in aluminium foil, placed inside separate sterile tubes and stored at -80°C in an ultralow temperature basic upright freezer, (Thermo Fisher Scientific India Pvt.td, Mumbai, India) (Figure: 20) until further analysis of PTX-3 by ELISA.

ELISA Analysis for GCF Samples:

Prior to use, the contents of the kit and the samples were allowed to warm naturally to room temperature for 30 minutes. Standard solutions were prepared (Figure: 25). 50µL of each standard solution was dispensed into the wells leaving the first well blank. 38µL of the diluent and 2µL of the sample (Figure: 26) were added to the test well in sequence following which 50µL of streptavidin-horseradish peroxidase was added. The plate was then incubated at 37ºC for 1 hour (Figure: 27 & 28). Following incubation, the plate was washed 5 times using wash buffer and blotted onto paper towels. 50µL of substrate A and B were added to each well following which the plate was covered in aluminium foil and incubated at 37ºC for 10 minutes (Figure: 29). 50µL of the stop solution was then added to each well resulting in colour change from blue to yellow (Figure: 30). Finally, the optical densities were read at 405nm by a microplate reader (Figure: 31). A standard curve was obtained (Figure: 32). The samples were compared with the standards. The concentrations of PTX-3 were expressed as nanograms per millilitre (ng/mL).

DEPARTMENT OF PERIODONTICS

LEVELS OF PENTRAXIN-3 IN GINGIVAL CREVICULAR FLUID OF PATIENTS WITH AGGRESSIVE AND CHRONIC PERIODONTITIS- AN IN VIVO STUDY

PROFORMA

FORM I - SCREENING PROFORMA

NAME: O.P NO:

AGE: SEX:

OCCUPATION:

POSTAL ADDRESS:

TELEPHONE NUMBER:

INCLUSION CRITERIA:

Group 1: Healthy individuals (Control group)

- Absence of bleeding on probing.
- Probing depth (PD) < 3mm.
- Absence of clinical attachment loss.
- Patients without any systemic disease.

Group 2: Chronic Periodontitis (Test group I)

- Localized (or) generalized mild, moderate or severe chronic periodontitis patients (According to criteria given by Armitage)

Group 3: Aggressive Periodontitis (Test group II)

- Localized (or) generalized aggressive periodontitis patients – (According to Lang's criteria)

EXCLUSION CRITERIA:

- Patients with history of antibiotic and anti-inflammatory drug intake in the past 3 months.
- Patients who have undergone any form of periodontal therapy in past 6 months.
- Patients with systemic disease that may have an impact on the levels of Pentraxin-3
- Pregnant/Lactating women

FORM II- HISTORY PROFORMA

Chief complaint with duration:

	Absent	**Present**
1. Bleeding gums	☐	☐
2. Bad breath	☐	☐
3. Pain in gums	☐	☐
4. Swollen gums	☐	☐
5. Pus discharge from gums	☐	☐
6. Mobility	☐	☐
7. Hypersensitivity	☐	☐

8. Any other complaint's (Specify):

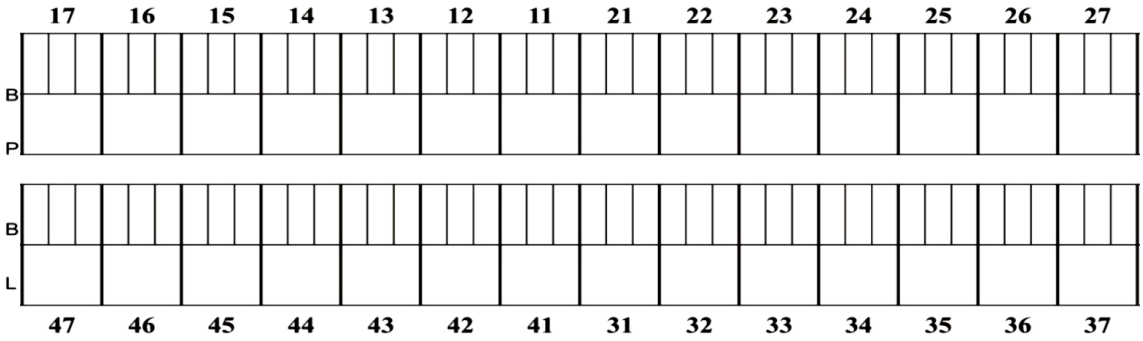
PERSONAL HISTORY:

1. Brushing habit:

FORM III- CLINICAL ASSESSMENT

DATE: _____

1. PLAQUE INDEX (SILNESS AND LOE 1967):



Calculation:

$$\frac{\text{Sum of score of each tooth}}{\text{Total number of teeth examined}}$$

Inference:

Excellent: 0

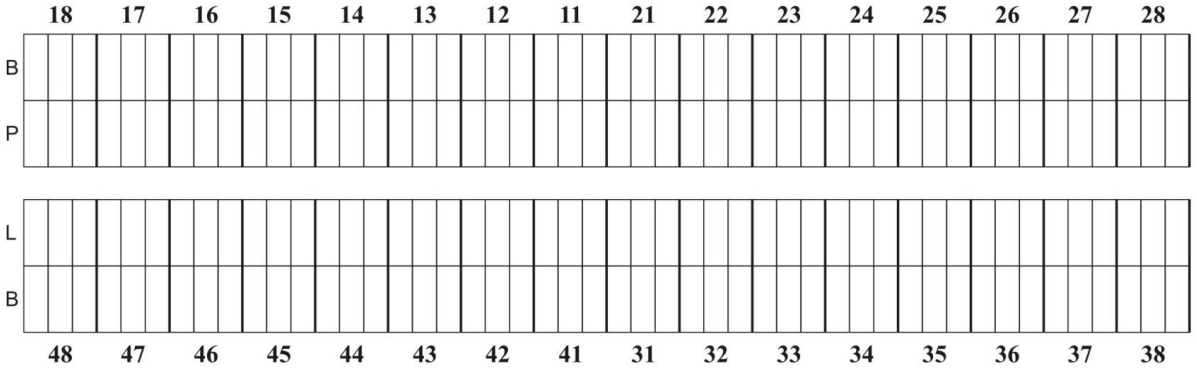
Good: 0.1 – 0.9

Fair: 1.0 – 1.9

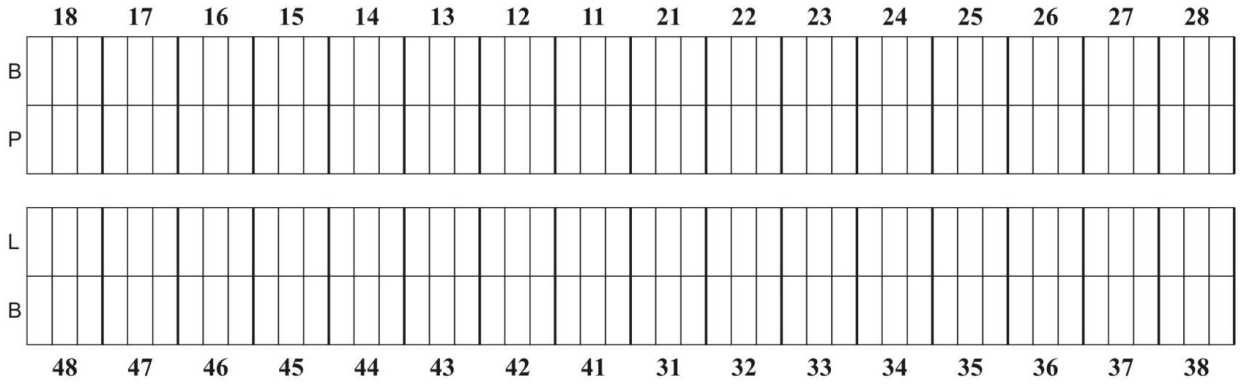
Poor: 2.0-3.0

2. PERIODONTAL STATUS

a) PROBING DEPTH (CONVENTIONAL PROBING METHOD)



b) CLINICAL ATTACHMENT LEVEL:



2) MODIFIED SULCULAR BLEEDING INDEX (Mombelli et. al, 1987)

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**

$$\text{Bleeding on Probing \%} = \frac{\text{Total score obtained}}{\text{Maximum score obtained} \times \text{Total number of teeth examined}} \times 100\%$$

FORM IV- LABORATORY INVESTIGATION

Levels of Pentraxin-3 as measured by enzyme linked immunosorbent assay

CONSENT FORM

CERTIFICATE BY INVESTIGATOR

I certify that I have disclosed all details about the study in terms easily understood by the patient.

Dated: _____ **Signature: _____**

Name: _____

CONSENT BY SUBJECT

I have been informed to my satisfaction, by the attending dentist, the purpose of the clinical examination and the laboratory investigation in a language understood by me.

I, exercising my free power of choice, hereby, give my consent to be included as a subject in the study "**LEVELS OF PENTRAXIN-3 IN GINGIVAL CREVICULAR FLUID OF PATIENTS WITH AGGRESSIVE AND CHRONIC PERIODONTITIS- AN IN VIVO STUDY"**

Dated: _____ **Signature or thumb impression**

ஒப்புதல் படிவம்

நான் புரிந்து கொள்ளக்கூடிய மொழியில் என்னை பரிசோதிக்கும் பல் மருத்துவரால், இந்த மருத்துவ பரிசோதனை மற்றும் ஆய்வக ஆய்வுக்கான காரணத்தை தெரிவிக்கப்பட்டுள்ளேன்.

இந்த ஆய்வில் "LEVELS OF PENTRAXIN-3 IN GINGIVAL CREVICULAR FLUID OF PATIENTS WITH AGGRESSIVE AND CHRONIC PERIODONTITIS- AN *IN VIVO* STUDY" பங்கேற்க, நான் முழு மனதுடன் சம்மத்திக்கிறேன்.

தேதி

கையெழுத்து அல்லது கை எண்ணம்

Figure 9: Armamentarium for GCF collection

Figure 10: Chronic Periodontitis- Clinical image

Figure 11: Chronic Periodontitis- Radiographic image

Figure 12: Chronic Periodontitis- GCF collection

Figure 13: Aggressive Periodontitis- Clinical image

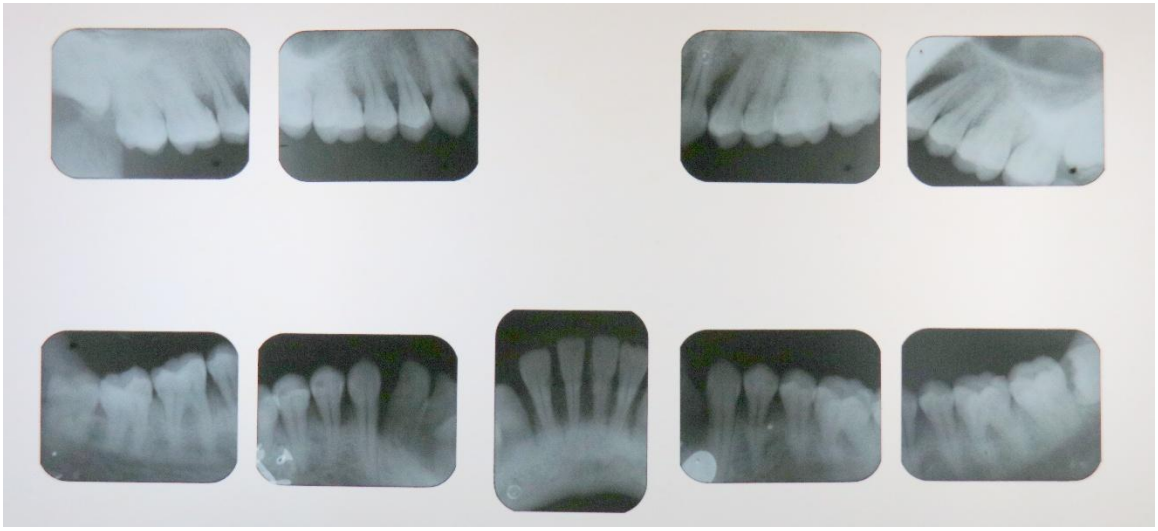


Figure 14: Aggressive Periodontitis- Radiographic image

Figure 15: Aggressive Periodontitis- Collection of GCF

Figure 16: Healthy subject- Clinical image

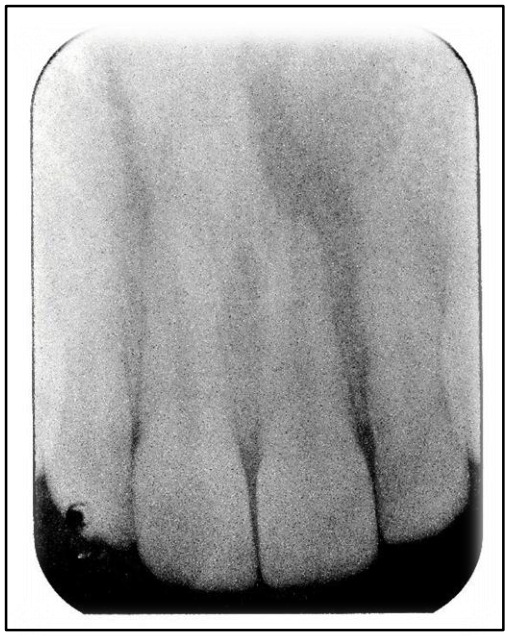


Figure 17: Healthy subject- Radiographic image

Figure 18: Healthy subject- Collection of GCF

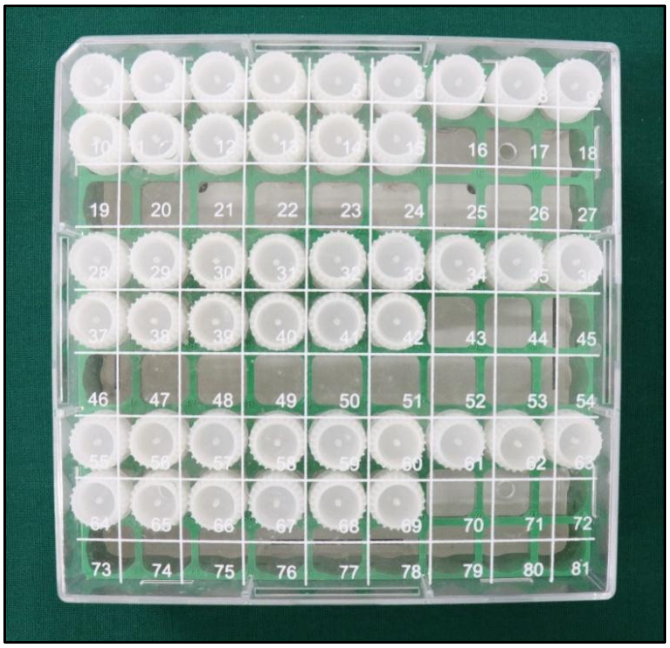


Figure 19: Samples arranged and stored in cryogenic storage box

Figure 20: Ultra- low temperature freezer for storage of samples

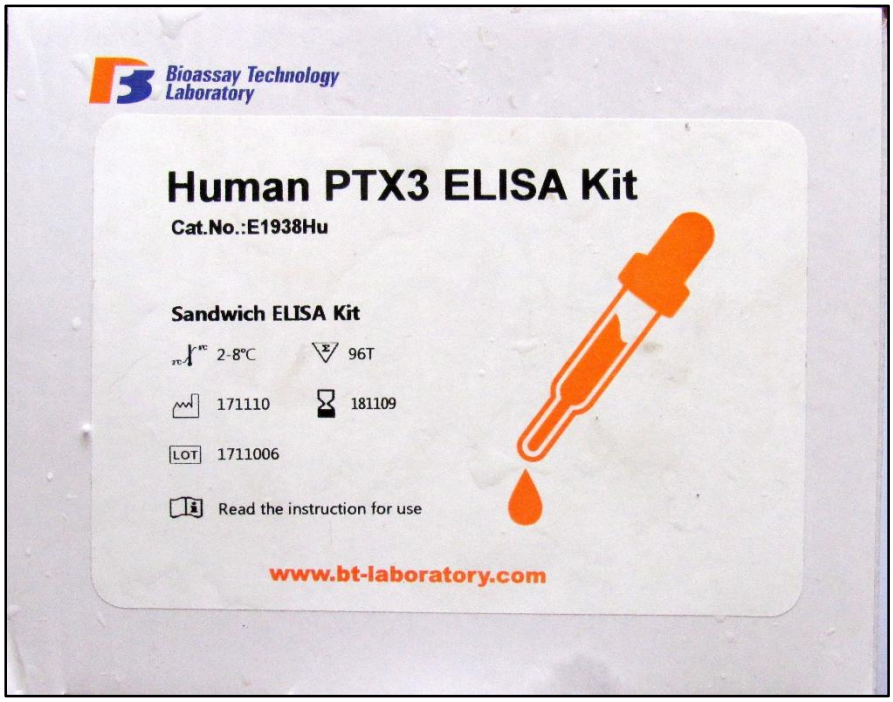


Figure 21: ELISA kit for PTX-3

Figure 22: Pre coated plate supplied with the ELISA KIT

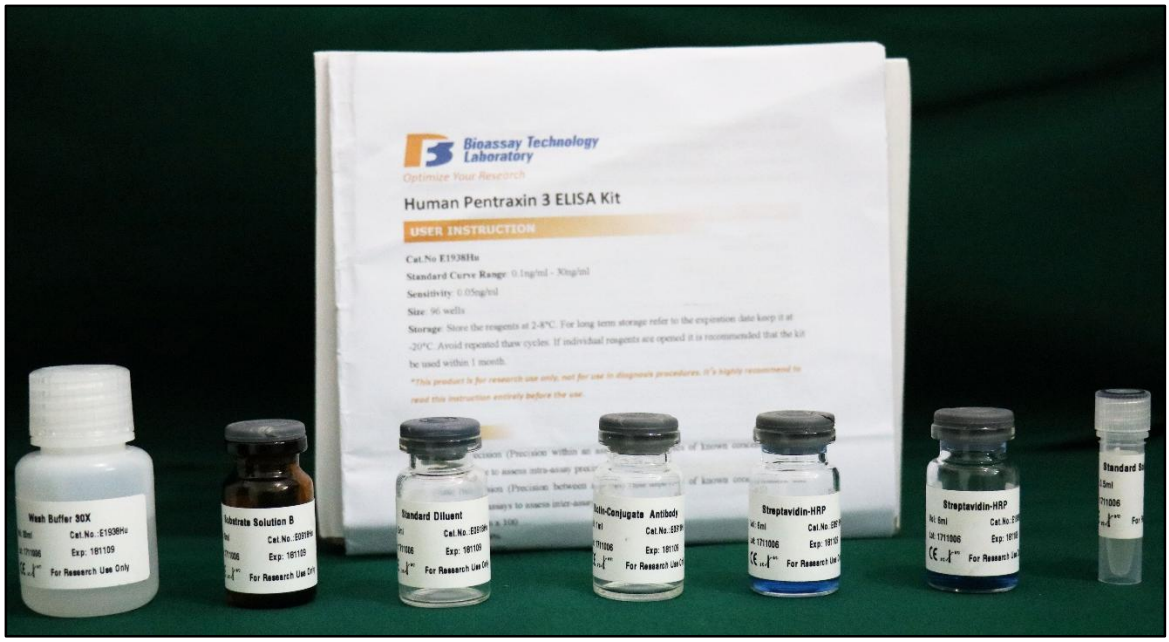


Figure 23: Reagents and manual supplied with the ELISA kit

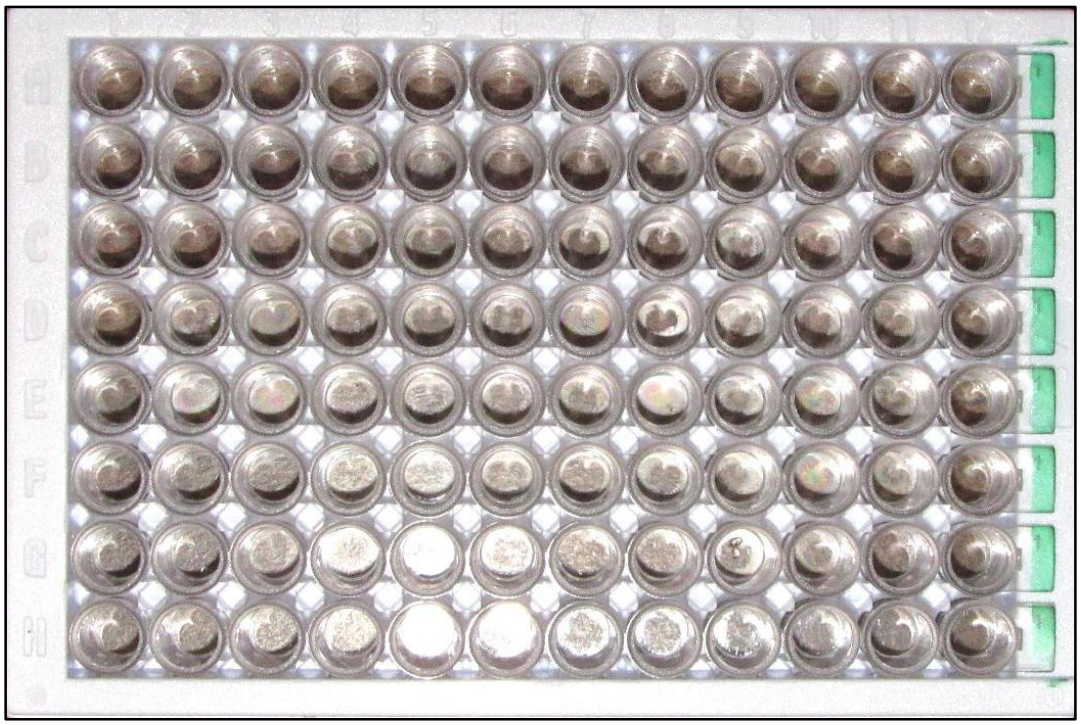


Figure 24: ELISA plate with 96 wells

Figure 25: Preparation of standard solutions

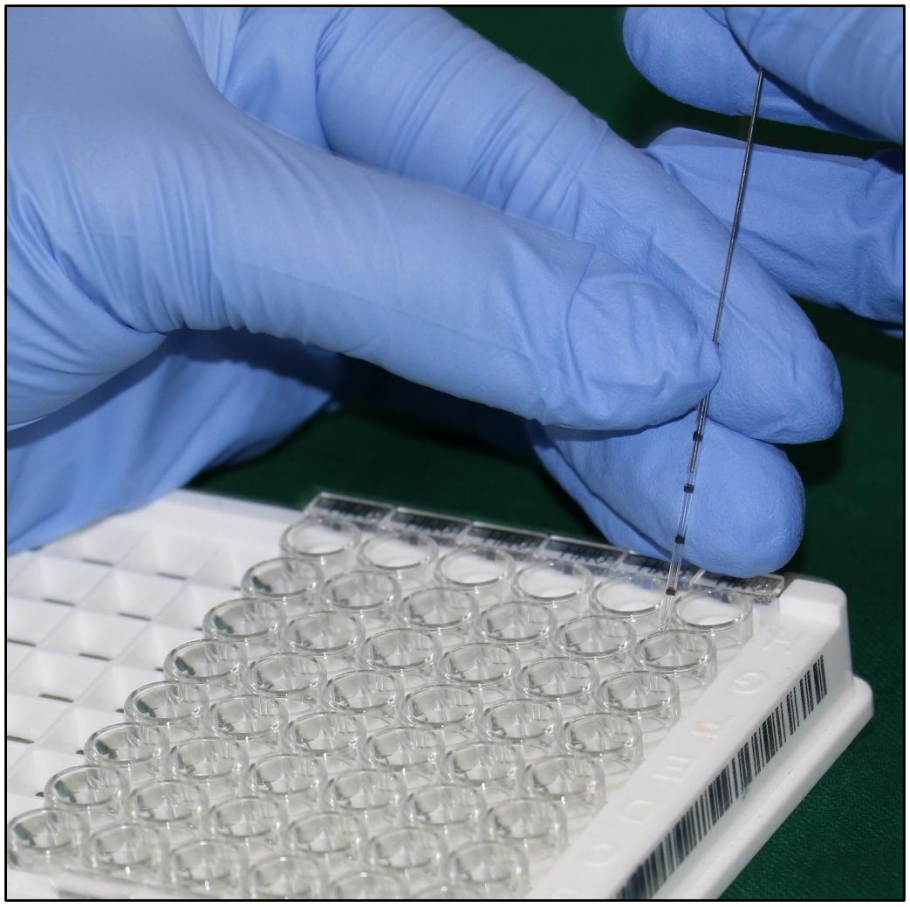


Figure 26: Dispensing of GCF samples into the pre-coated wells

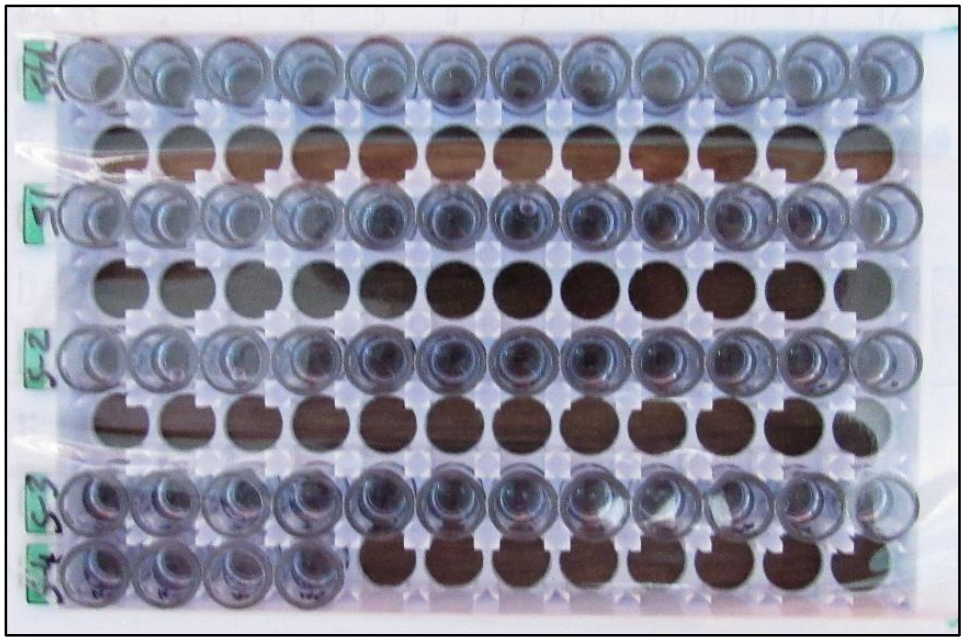


Figure 27: Sealed ELISA plate following addition of streptavidin- HRP

Figure 28: Incubation for 1 hour at 37ºC

Figure 29: Wrapping with aluminium foil for incubation following washing with buffer and addition of substrates

Figure 30: Colour change observed following addition of stop solution after incubation in the dark for 10 minutes at 37ºC

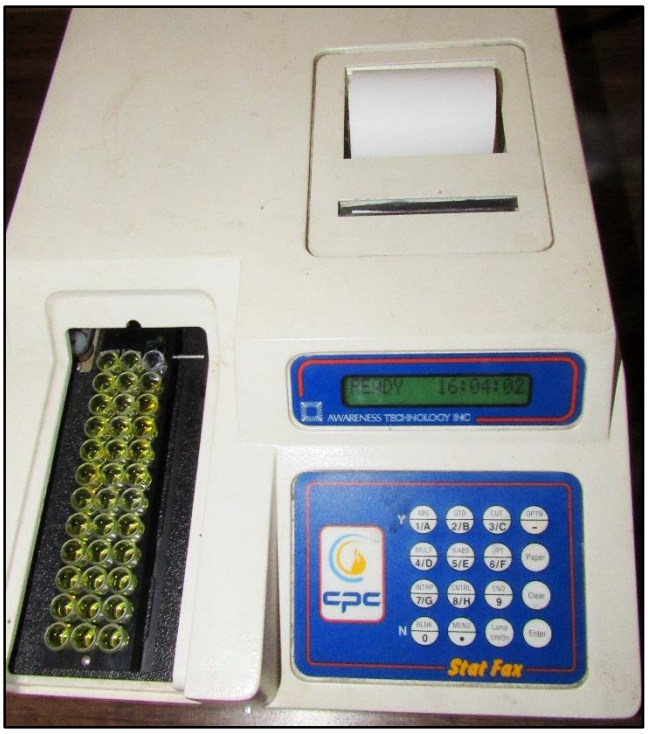


Figure 31: Optical density measured using a microplate reader

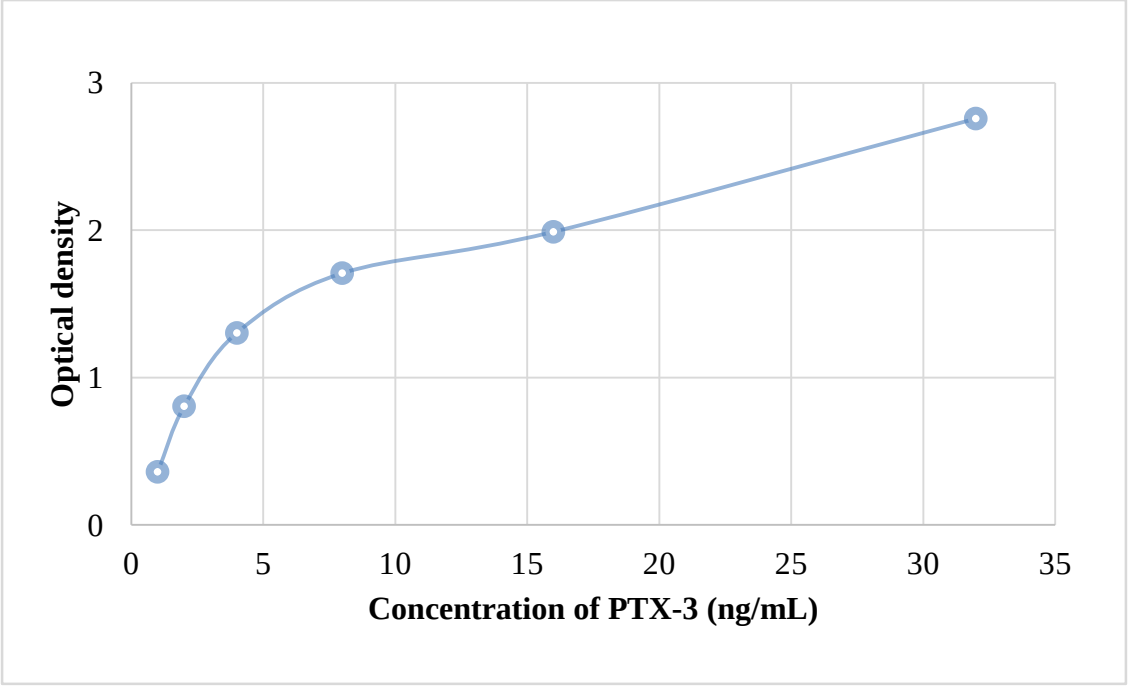


Figure 32: Standard curve

Statistical analysis:

The sample size, as calculated using sampling software - G.Power Version 3.1.9.2 with an alpha error of 5% and power of 95%, was found to be 15 in each group. Statistical analysis was performed using a commercially available software (SPSS 16.0; SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was used to investigate whether the data were normally distributed. Variables in the 3 groups were compared using one-way analysis of variance (ANOVA) and for multiple comparisons Tukey HSD was used. Pearson correlation was used to assess the correlation between the levels of PTX-3 and the clinical periodontal parameters.

Demographic data

The mean age of subjects in the CP, AgP and H groups were; 41.53, 27.93 and 22.20 years respectively with a statistically significant difference between the groups ($p < 0.001$) (Table: 4). The gender distribution in the groups was 11 males and 4 females in the CP group, 7 males and 8 females in the AgP group and 2 males and 13 females in the H group. Both CP and AgP groups included 2 localized and 13 generalized cases (Table: 4).

Clinical periodontal parameters and PTX-3 levels- intergroup comparison:

The clinical parameters and PTX-3 levels in the study groups were compared by means of one way ANOVA (Tables: 5 & 6, Graphs: 1-7). The mean full mouth probing depth (fPD) of CP, AgP and that of H groups were 3.76mm, 4.56mm and 1.58mm respectively. The difference between the groups was statistically significant ($p < 0.001$) (Table: 5, Graph: 1). The mean full mouth clinical attachment loss (fCAL) was 4.01mm, 4.54mm and 0.0mm in CP, AgP and H groups respectively with the difference between the

groups being statistically significant ($p < 0.001$) (Table: 5, Graph: 2). The full mouth plaque index (fPI) levels were 0.91, 1.04 and 0.32 respectively for subjects belonging to CP, AgP and H groups and the difference between the groups was statistically significant ($p < 0.001$) (Table: 5, Graph: 3). The mSBI demonstrated mean values of 53.20%, 75.87% and 0% respectively for CP, AgP and H groups, with a statistically significant intergroup difference ($p < 0.001$) (Table: 5, Graph 4).The clinical parameters namely, PD, CAL and PI were compared for individual teeth (i.e, from the source of GCF collection) in order to determine if there was any trend observed on comparing the groups and levels of PTX-3. The individual PD (iPD) demonstrated mean values of 4.56mm, 5.60mm and 1.54mm in the CP, AgP and H groups respectively. The intergroup difference in iPD values was found to be statistically significant ($p < 0.001$) (Table: 5, Graph: 5). The mean individual CAL (iCAL) values for CP, AgP and H groups were 5.09mm, 5.78mm and 0.0mm respectively with the intergroup difference being statistically significant ($p < 0.001$) (Table: 5, Graph: 6). The individual PI (iPI) had values of 1.03, 0.75 and 0.35 in the CP, AgP and H groups respectively and the difference between the groups was statistically significant ($p < 0.05$) (Table: 5, Graph: 7). The levels of PTX-3 had mean values of 2.81ng/mL, 7.34ng/mL and 1.33ng/mL in the CP, AgP and H groups respectively, with the difference between the groups being statistically significant ($p < 0.001$) (Table: 6, Graphs: 8 & 9).

Pairwise comparison of the clinical parameters and PTX-3 levels between groups:

On pairwise comparison, the mean fPD was statistically significant and higher ($p = 0.010$) in the AgP than the CP group with a mean difference of 0.80mm; in the CP than in the H group with a mean difference of 2.18mm ($p < 0.001$) and in the AgP group than in H with a mean difference of 2.99mm ($p < 0.001$) (Table: 7).

Likewise, the mean iPD was statistically significant and greater in the AgP than in the CP group ($p < 0.01$); in the CP than in the H ($p < 0.001$) and in the AgP than in the H group ($p < 0.001$) with mean differences of 1.05mm, 3.01mm and 4.06mm respectively (Table: 8).

On pairwise comparison, the difference in mean fCAL (0.53mm) was not found to be statistically significant between the AgP and CP groups ($p > 0.05$). However, the fCAL values were greater and statistically significant in the CP group when compared to the H group ($p < 0.001$) and in the AgP group when compared to the H group ($p < 0.001$) with mean differences of 4.01mm and 4.54mm respectively (Table: 9).

Similarly, mean iCAL did not demonstrate any statistically significant difference ($p > 0.05$) between the AgP and CP groups with a mean difference of 0.69mm. However the values were significantly higher in the CP and AgP groups when compared individually to the H group with mean differences of 5.09mm and 5.78mm respectively and $p < 0.001$ on both occasions. (Table: 10).

On comparing the fPI, the mean difference (0.13) between AgP and CP group was not found to be statistically significant ($p > 0.05$). However the difference between CP and H (0.59) as well as that between AgP and H (0.72) was statistically significant with $p < 0.001$ on both counts (Table: 11).

The iPI demonstrated no statistically significant difference between the AgP and CP groups (mean difference: -0.28, $p > 0.05$) and between the AgP and H groups (mean difference: 0.40, $p > 0.05$). However a significant difference was observed between the CP and H groups (mean difference: 0.68, $p < 0.01$) (Table: 12).

The mSBI demonstrated greater values in the AgP group with the difference between the AgP and CP groups (22.67%) being statistically significant ($p < 0.001$). Likewise, the difference between CP and H (53.20%) as well as that in AgP and H (75.87%) groups was found to be statistically significant with $p < 0.001$ on both comparisons (Table: 13).

The GCF levels of PTX-3 was found to be highest in the AgP group with the difference between the AgP and CP groups being 4.53ng/mL, between the CP and H groups being 1.48ng/mL and that between the AgP and H groups being 6.01ng/mL with $p < 0.001$ on all comparisons indicating that the differences were statistically significant (Table: 14).

Pearson correlation:

The levels of PTX-3 were checked for their correlation with clinical periodontal parameters namely; fPD, iPD, fCAL, iCAL, fPI, iPI and mSBI. The levels of PTX-3 correlated positively in each case with the correlation between PTX-3 and fPD ($r = 0.708$), PTX-3 and iPD ($r = 0.704$), PTX-3 and fCAL ($r = 0.687$), PTX-3 and iCAL ($r = 0.643$), PTX-3 and fPI ($r = 0.495$) and PTX-3 and mSBI ($r = 0.752$) being of statistical significance, having a p value of < 0.001 in each case. Though age ($r = 0.013$) and iPI ($r = 0.071$) demonstrated a positive correlation with PTX-3 levels, this correlation was not statistically significant ($p > 0.05$) in both cases (Table: 15, Graph: 10).

Table 4: Demographic data

	CP	AgP	H
Number of subjects (n)	15 (13 generalized and 2 localized)	15 (13 generalized and 2 localized)	15
Age (years) mean±SD	41.5333±0.980428	27.9333±5.96977	22.2±1.69874
Gender Male Female	 11 4	 7 8	 2 13

Table 5: Clinical parameters and levels of PTX-3 in the study subjects- one way ANOVA

	CP (n = 15)	AgP (n = 15)	H (n = 15)	p value
fPD (mm) mean±SD	3.7561±0.66574	4.5605±1.00467	1.5758±0.26553	<0.001
fCAL (mm) mean±SD	4.0085±0.75293	4.5367±1.28862	0.0±0.0	<0.001
fPI mean±SD	0.9095±0.36581	1.0389±0.58232	0.3208±0.17313	<0.001
mSBI (%) mean±SD	53.1954±16.76909	75.8714±19.02503	0.0±0.0	<0.001
iPD (mm) mean±SD	4.5556±1.08866	5.6000±0.98159	1.5444±0.41051	<0.001
iCAL (mm) mean±SD	5.0889±1.40134	5.7778±1.75443	0.0±0.0	<0.001
iPI mean±SD	1.0333±0.71256	0.7500±0.68791	0.3500±0.31053	0.012

Table 6: Level of PTX-3 among the study groups

	CP	**AgP**	**H**	**p value**
Level of PTX-3 (ng/mL) mean±SD	2.8067±0.54046	7.3400±1.27772	1.3267±0.54703	<0.001

Table 7: Pairwise comparison of fPD between groups using Tukey HSD

Variable	**Groups being compared**	**Mean difference (mm)**	**p value**
fPD (mm)	AgP Vs CP	0.80439	0.010
	CP Vs H	2.18024	<0.001
	AgP Vs H	2.98463	<0.001

Table 8: Pairwise comparison of iPD between groups using Tukey HSD

Variable	**Groups being compared**	**Mean difference (mm)**	**p value**
iPD (mm)	AgP Vs CP	1.04445	0.006
	CP Vs H	3.0112	<0.001
	AgP Vs H	4.05557	<0.001

Table 9: Pairwise comparison of fCAL between groups using Tukey HSD

Variable	**Groups being compared**	**Mean difference (mm)**	**p value**
fCAL (mm)	AgP Vs CP	0.52821	0.225
	CP Vs H	4.00849	<0.001
	AgP Vs H	4.53671	<0.001

Table 10: Pairwise comparison of iCAL between groups using Tukey HSD

Variable	Groups being compared	Mean difference (mm)	p value
iCAL (mm)	AgP Vs CP	0.68889	0.323
	CP Vs H	5.08889	<0.001
	AgP Vs H	5.77778	<0.001

Table 11: Pairwise comparison of fPI between groups using Tukey HSD

Variable	Groups being compared	Mean difference	p value
fPI	AgP Vs CP	0.12933	0.665
	CP Vs H	0.58873	<0.001
	AgP Vs H	0.71805	<0.001

Table 12: Pairwise comparison of iPI between groups using Tukey HSD

Variable	Groups being compared	Mean difference	p value
iPI	AgP Vs CP	-0.2833	0.406
	CP Vs H	0.68333	0.009
	AgP Vs H	0.40000	0.173

Table 13: Pairwise comparison of mSBI between groups using Tukey HSD

Variable	Groups being compared	Mean difference (%)	p value
mSBI	AgP Vs CP	22.67605	<0.001
	CP Vs H	53.19535	<0.001
	AgP Vs H	75.87140	<0.001

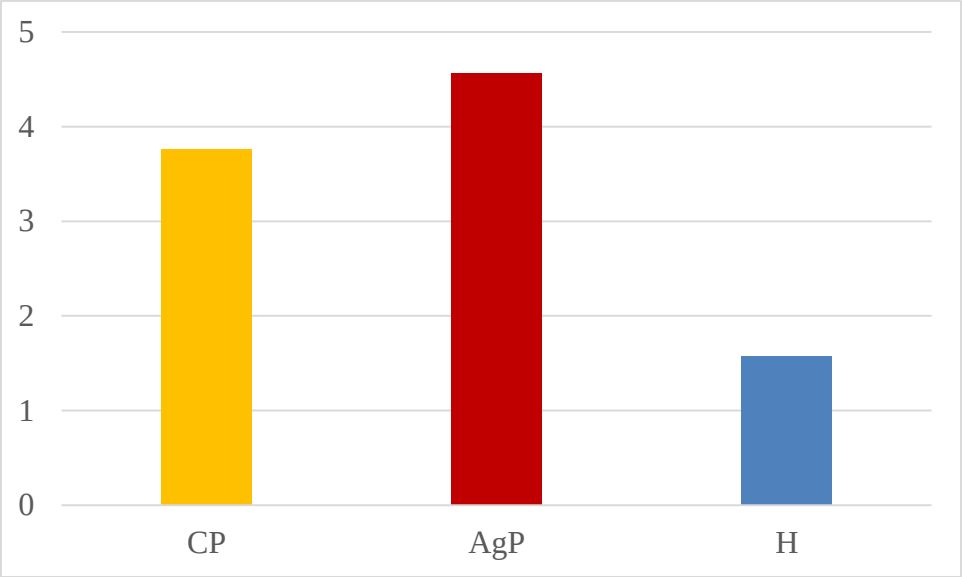
Table 14: Pairwise comparison of PTX-3 between groups using Tukey HSD

Variable	Groups being compared	Mean difference	p value
PTX-3	AgP Vs CP	4.53333	<0.001
	CP Vs H	1.48000	<0.001
	AgP Vs H	6.01333	<0.001

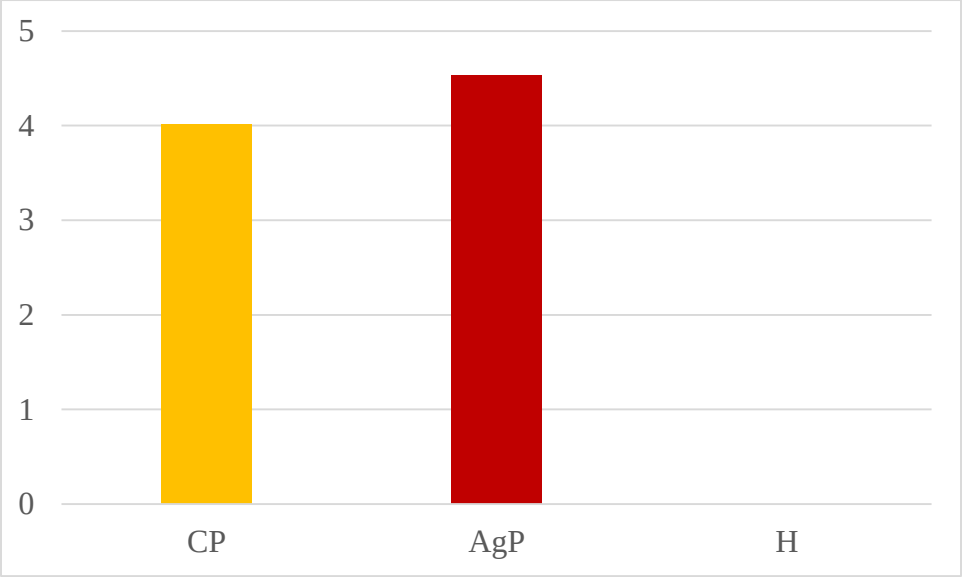
Table 15: Overall Pearson correlation

Level of PTX-3	Parameter	Pearson Correlation (r)	p value
	Age	0.013	0.933
	fPD	0.708	<0.001
	iPD	0.704	<0.001
	fCAL	0.687	<0.001
	iCAL	0.643	<0.001
	fPI	0.495	<0.001
	iPI	0.071	0.645
	mSBI	0.752	<0.001

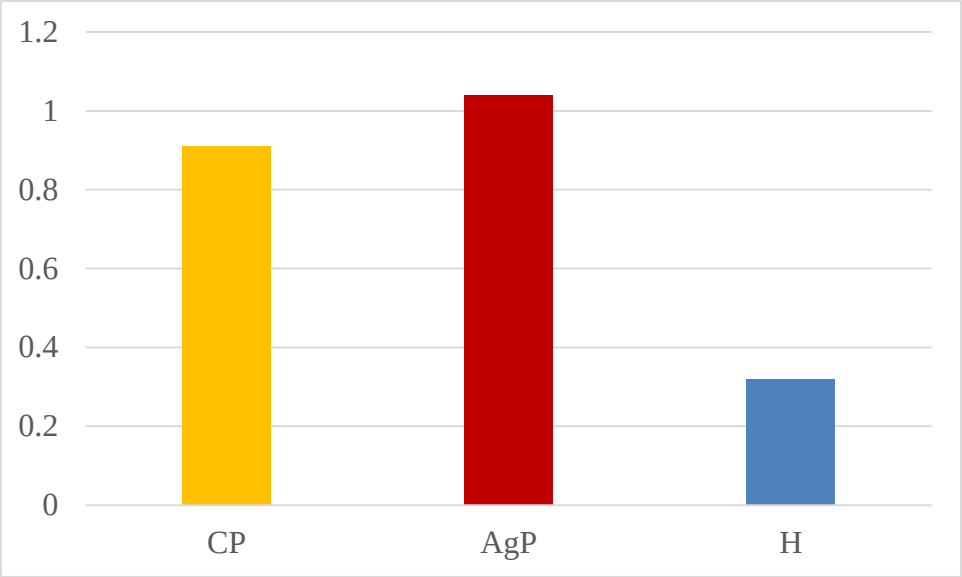
Graph 1: Intergroup comparison of fPD using one way ANOVA



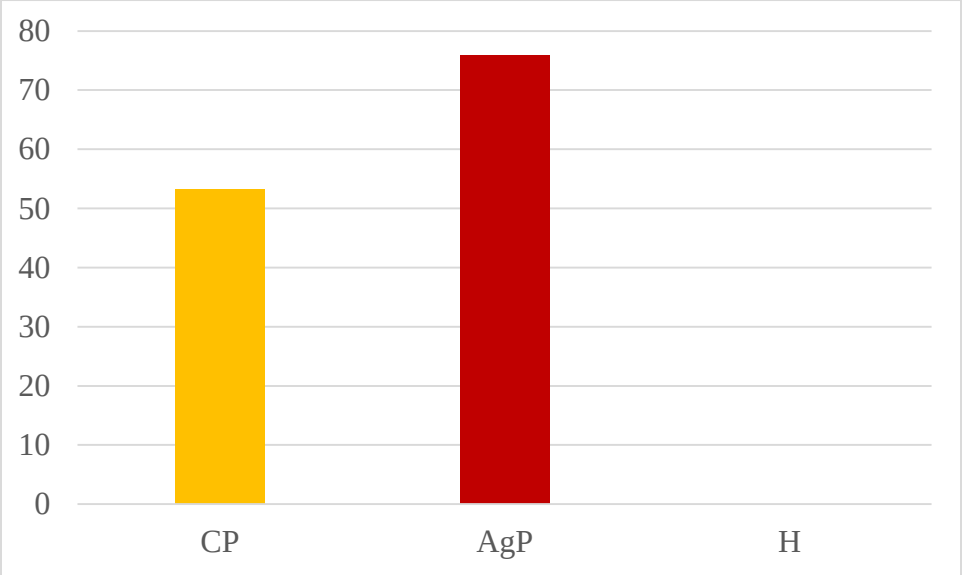
Graph 2: Intergroup comparison of fCAL using one way ANOVA



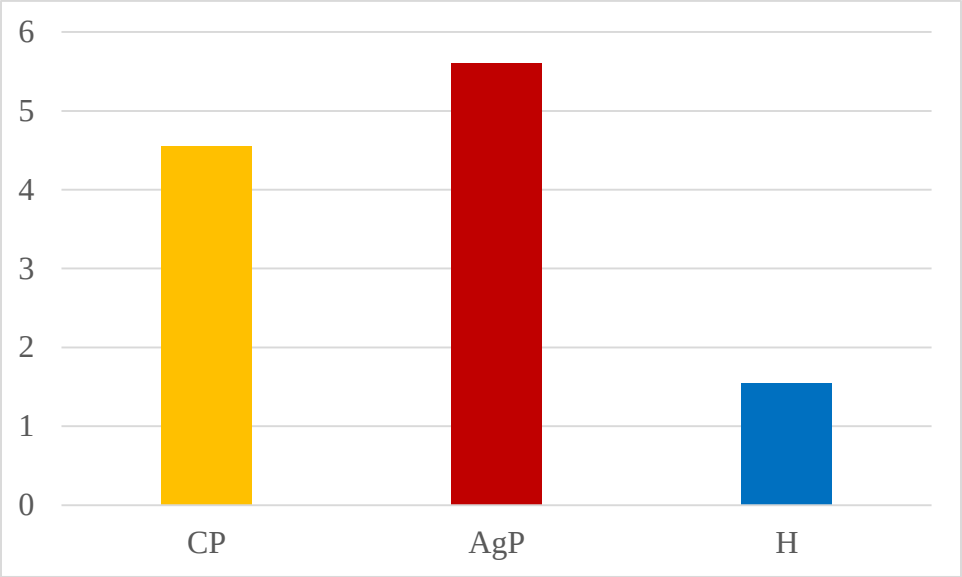
Graph 3: Intergroup comparison of fPI using one way ANOVA



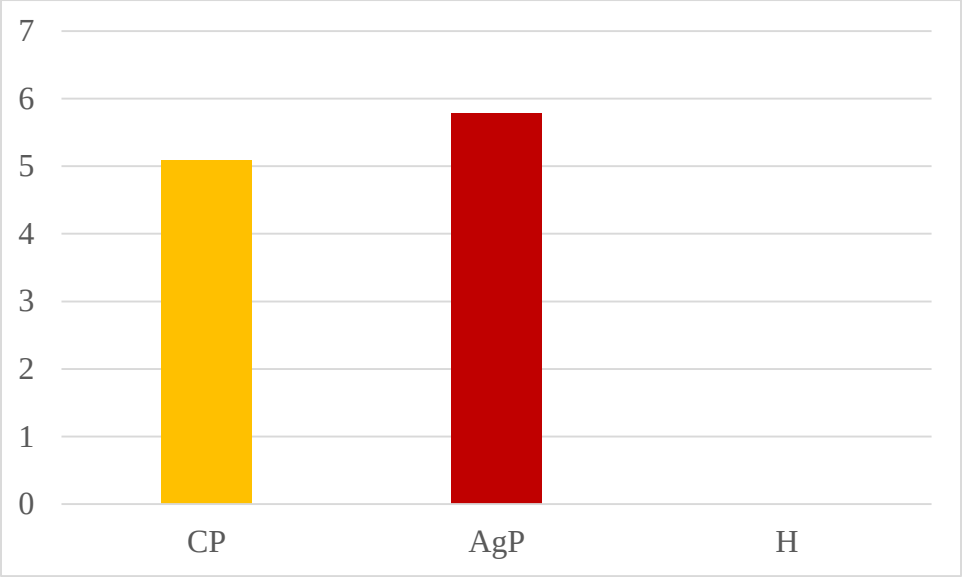
Graph 4: Intergroup comparison of mSBI using one way ANOVA



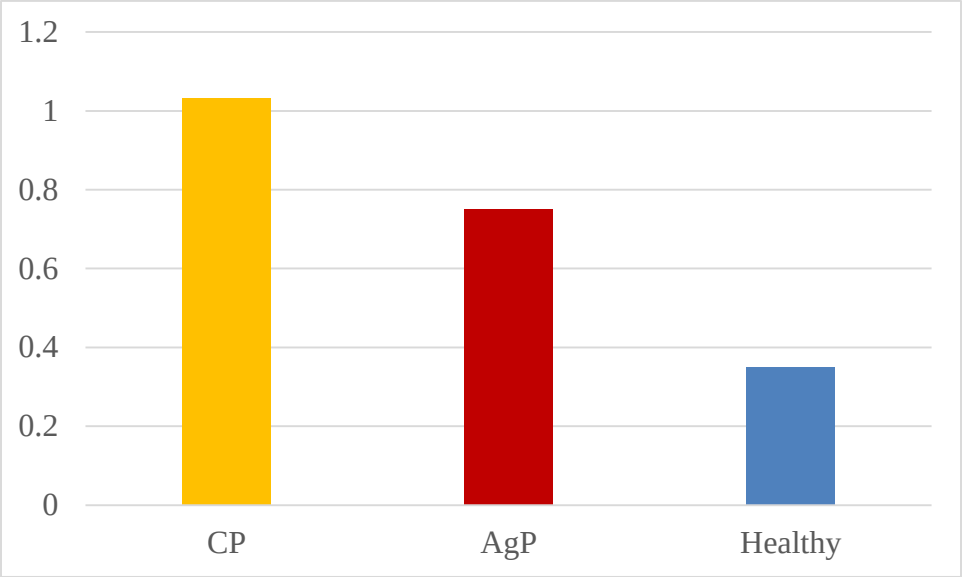
Graph 5: Intergroup comparison of iPD using one way ANOVA



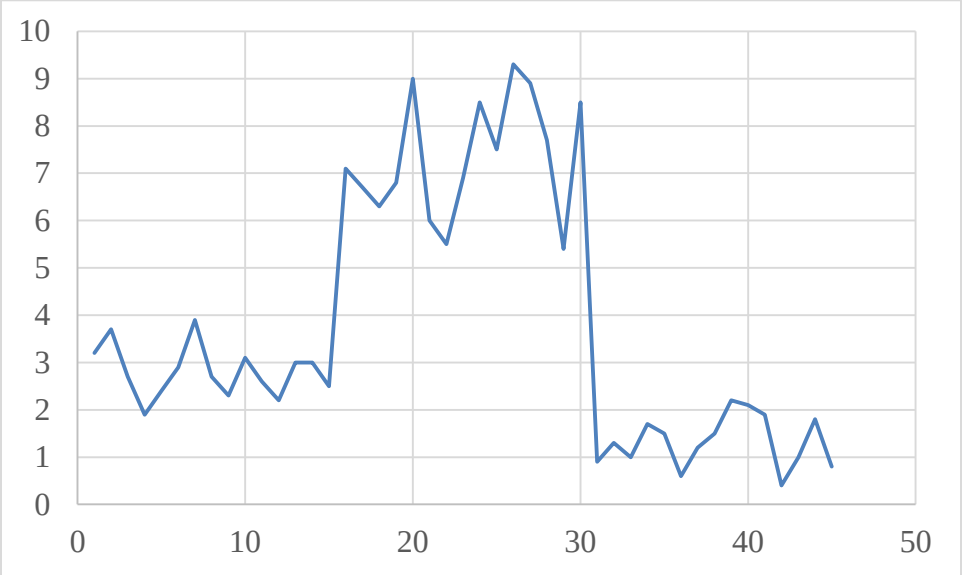
Graph 6: Intergroup comparison of iCAL using one way ANOVA



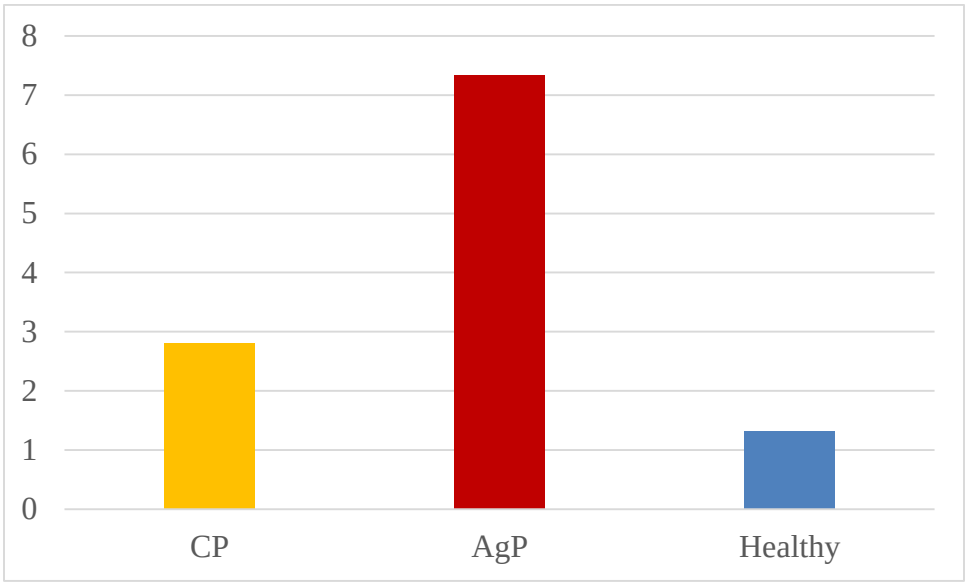
Graph 7: Intergroup comparison of iPI using one way ANOVA



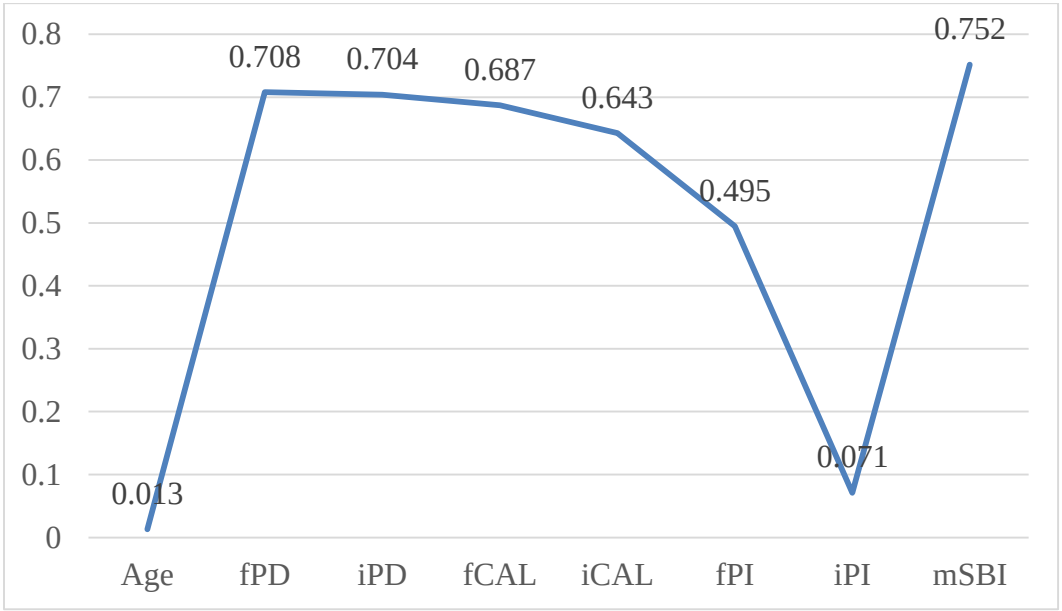
Graph 8: Scatter plot depicting the levels of PTX-3 (in order 1-15 CP, 16- 30 AgP and 31-45 H)



Graph 9: Intergroup comparison of PTX-3 levels using one way ANOVA



Graph 10: Overall Pearson correlation



The present cross- sectional study compared the GCF levels of PTX-3 in CP, AgP and in H subjects. A total of 45 subjects were included in this study with 15 participants in each group (15 each in CP, AgP and H group). This sample size was determined to be appropriate statistically and was arrived at before commencing the study. The levels of PTX-3 were detected following the collection of all samples by means of ELISA. Statistical analysis was then performed to compare the levels of PTX-3 and the clinical parameters in the study groups. To the best of our knowledge, this was the first study comparing GCF levels of this acute phase protein in the above mentioned groups.

Unlike other members of the PTX family, PTX-3 is produced extrahepatically by various cell types (monocytes, macrophage, PMN, endothelial cells, dendritic cells, fibroblasts, epithelial cell etc.). This results in quick elevation of this protein in response to an insult. The structure of PTX-3 demonstrates little variation amongst species. The conservation of the structure of PTX-3 through the process of evolution hints that this acute phase protein is an essential component of the immunoinflammatory response. PTX-3 has various functions, of particular interest being its dual role in the host immune-inflammatory response. It can lead to elevation of pro-inflammatory cytokines and neutrophilic infiltration in certain conditions, at the same time it also has a protective role. It protects against infection by facilitating phagocytosis via complement. PTX-3 helps in clearing neutrophilic infiltration and protects against LPS mediated damage. It also helps in pathogen recognition and opsonization, complement activation, release of cytokines, maturation of immune cells and activation of glycosylation dependent inflammatory responses. In addition, PTX-3 increases the tolerance to host antigens by preventing cross presentation of antigens. In vitro and in vivo studies have shown that this protein has a

dampening effect on the neutrophils, thereby reducing the neutrophil mediated destruction.[132] These findings when extrapolated to the periodontium, suggest that PTX-3 may be of great importance in maintaining the balance between destruction and repair.

It has been claimed that due to its local production, PTX-3 is site specific, with the levels being the highest in areas of active inflammation. In order to explore this claim, clinical periodontal parameters namely; fPD, iPD, fCAL, iCAL, fPI, iPI and mSBI were evaluated to study the correlation. These parameters have been traditionally used to arrive at a periodontal diagnosis. In the test groups (CP and AgP), the teeth with the greatest clinically discernible activity as evidenced by bleeding on probing were selected as a source of GCF collection.

The PD, CAL and PI scores were calculated separately (iPD, iCAL and iPI) to check for an association at the individual tooth level. Almost all the teeth (in the test groups) that were selected for GCF collection, demonstrated bleeding scores of '3' (mSBI - **Mombelli *et al*. 1987**)[182] hence, an individual comparison of mSBI was not performed. Though bleeding on probing has been considered as the most reliable clinical marker of disease activity, the selected sites (in the test groups), all with the same bleeding score, demonstrated a variation in the levels of PTX-3, suggesting that, the degree of disease activity varies at the biochemical level.

On comparing the clinical periodontal parameters between the test groups, fPD was highest in the AgP group (4.57±1.00mm) followed by the CP (3.76±0.67mm) and H (1.58±0.27mm) groups with the intergroup difference being statistically significant ($p < 0.001$). On pairwise comparison of the same, the difference between the AgP and CP

groups (0.80mm) was found to be statistically significant (p = 0.01). On comparing the AgP and H groups and CP and H groups, the mean differences were 2.99mm and 2.18mm respectively with p < 0.001 on both comparisons. These results are in accordance with the study done by **Lakshmanan *et al*. (2014)[164]** who demonstrated mean fPD values of 4.58±1.18mm and 3.89±0.76mm in the AgP and CP groups respectively. No parallels could be drawn regarding statistical significance due to the lack of data on the same. In contrast, **Gümüş *et al.* (2014)[162]** reported no statistically significant difference in the PD between CP (5.20mm) and AgP (5.00mm) groups. However, the PD in the periodontitis groups (CP and AgP) were greater and statistically significant than the control groups (2.00mm) with p < 0.05.

The iPD demonstrated higher values in the AgP group (5.60±0.98mm) followed by the CP (4.56±1.09) and the H groups (1.54±0.41mm). The intergroup difference was found to be statistically significant (p < 0.001). On pairwise comparison, the mean difference in iPD (4.06mm) was greatest and statistically significant (p < 0.001) between the AgP and H groups. The difference between the CP and H groups (3.01mm) was also found to be of statistical significance (p < 0.001). Likewise comparison between the AgP and CP groups demonstrated a mean difference of 1.05mm in iPD, which was statistically significant (p < 0.01). These results are in accordance with the study by **Lakshmanan *et al*. (2014)**,[164] where the iPD was higher in the AgP (6.32±1.36mm) than in the CP group (5.66±1.18mm), with the values being the least in the H group (1.17±0.15mm). As data regarding statistical significance were not available, parallels could not be drawn with the statistical values obtained in our study.

The fCAL demonstrated highest mean values in the AgP group (4.01±0.75mm) followed by the CP group (4.54±1.29mm). The H group demonstrated no attachment loss. The intergroup difference was found to be statistically significant ($p < 0.001$). On pairwise comparison between the AgP and the CP groups, the mean difference in the fCAL was 0.53mm and this difference was not statistically significant ($p > 0.05$). However, the pairwise comparison between the AgP and the H group (mean difference: 4.54mm) as well as that between the CP and H groups (mean difference: 4.01mm) demonstrated a statistically significant difference with $p < 0.001$ on both comparisons. These results were similar to those obtained by **Lakshmanan *et al.* (2014)[164]** who demonstrated a similar mean difference in CAL of 0.50mm with the values being higher in the AgP group. However, no statistical comparisons could be made with our study due to the lack of data on statistical significance. **Gümüş *et al.* (2014)[162]** also found no statistically significant difference in the CAL values between the AgP and the CP groups (mean difference: 0.5mm, $p > 0.05$), however, the values were higher in the CP than in the AgP group.

The iCAL showed highest values in the AgP group (5.78±1.75mm) followed by the CP group (5.09±1.40mm). The H group demonstrated no attachment loss. The intergroup difference was found to be statistically significant ($p < 0.001$). Pairwise comparison of the AgP and the CP groups demonstrated a mean difference of 0.69mm which was not statistically significant ($p > 0.05$). Comparison between the AgP and H group and that between the CP and H group, demonstrated mean differences of 5.78mm and 5.09mm respectively which was found to be statistically significant in both cases ($p < 0.001$). These results are similar to those obtained by **Lakshmanan *et al*. (2014)**,[162] who demonstrated a difference of 0.73mm between AgP and CP groups, with the values being higher in the

AgP group. This study could not be compared with our study at the statistical level due to the lack of data available on the same.

The fPI demonstrated values of 1.04±0.58, 0.91±0.37 and 0.32±0.17 in the AgP, CP and in the H groups respectively with a statistically significant difference being observed between the groups ($p < 0.001$). On pairwise comparison between the CP and AgP groups, no statistically significant difference was found (mean difference: 0.13, $p > 0.05$). The difference in fPI between the AgP and H groups (0.72) and that between the CP and H (0.59) group was found to be statistically significant with $p < 0.001$ in both cases. **Lakshmanan *et al.* (2014)**,[164] also demonstrated minimal difference (0.07) in the fPI values between the CP and AgP groups. **Ertugrul *et al*. (2017)**[183] similarly reported mean fPI values of 1.86±0.14 and 1.55±0.41 in AgP and CP groups respectively with a mean difference of 0.31. Though no statistical comparisons were made in these studies, the fPI values were marginally higher in the AgP group when compared to the CP group.

The mean iPI values were; 1.03±0.71, 0.75±0.69 and 0.35±0.31 in the CP, AgP and in the H groups respectively. On intergroup comparison, the difference between the 3 groups was found to be statistically significant ($p < 0.05$). On pairwise comparison, the difference observed between the CP and AgP groups (0.28) was not significant statistically ($p > 0.05$). On comparing the CP with the H group, difference in iPI value was found to be 0.68 and this difference was statistically significant ($p < 0.01$). The difference between the AgP and the H group (0.40) however, was not significant statistically ($p > 0.05$). **Lakshmanan *et al*. (2014)**[164] reported a difference of 0.06, 0.82 and 0.88 in iPI scores between the CP and AgP, between CP and H and between AgP and H groups respectively.

As with other clinical parameters, no data was available on statistical significance and hence, parallels could not be drawn with our study at the statistical level.

The mSBI demonstrated mean scores of; 53.20±16.77%, 75.87±19.03% and 0.0% in the CP, AgP and in the H groups respectively, with the AgP groups demonstrating the highest values and the intergroup difference being statistically significant ($p < 0.001$). On pairwise comparison, the difference between the AgP and CP groups was 22.68% which was of statistical significance ($p < 0.001$). The difference between the CP and H groups was, 53.20% which was statistically significant ($p < 0.001$). The difference between the AgP and the H group (75.87%) was also found to be statistically significant ($p < 0.001$). These results are in accordance with those obtained by **Suzuki *et al*. (2008)**,[184] who reported 65.3% and 72.6% mean bleeding in CP and in AgP subjects respectively. The difference (7.3%) between the groups (AgP and CP) however, was not statistically significant ($p > 0.05$). Likewise, **Baser et al. (2015)**[185] reported bleeding scores of 93.4±9.6% in the AgP and 88.5±15.5% in the CP group with a difference of 4.9% between the groups which was not statistically significant ($p > 0.05$).

The results obtained indicate that the GCF levels of PTX-3 increased with an increase in disease severity, being the least in the H subjects (1.33±0.54ng/mL) followed by that in the CP group (2.81±0.54ng/mL) and with the highest values being observed in the AgP group (7.34±1.28ng/mL). The intergroup differences were found to be statistically significant ($p < 0.001$). On pairwise comparison of PTX-3 levels between the AgP and CP groups, the mean difference (4.53ng/mL) was found to be statistically significant ($p < 0.001$). These results are in accordance with those obtained by **Lakshmanan *et al*. (2014)**[164] who demonstrated significantly higher levels of PTX-3 in gingival tissues of

subjects with AgP (8.35±5.08ng/mL) when compared to CP the (5.07±3.27ng/mL) and the H subjects (0.25±0.28ng/mL). The mean difference between the AgP and CP groups was 3.28ng/mL which was statistically significant ($p < 0.05$).

The results indicate that, overall, a positive correlation exists between clinical parameters namely; fPD ($r = 0.708$, $p < 0.001$), iPD ($r = 0.704$, $p < 0.001$), fCAL ($r = 0.687$, $p < 0.001$), iCAL ($r = 0.643$, $p < 0.001$), fPI ($r = 0.495$, $p = 0.001$) and mSBI ($r = 0.752$, $p < 0.001$). These results are in accordance with the study done by **Lakshmanan *et al*. (2014)[164]** who demonstrated a positive correlation between levels of PTX-3 and the clinical periodontal parameters. This finding suggests that the levels of PTX-3 increase with an increase in disease severity.

The results of the present study hence, suggest that PTX-3 has the potential to serve as a biomarker, indicating the sites of active disease, a feat that has been difficult to achieve clinically. The rate of destruction in AgP cases is faster than in CP indicating that these periodontal conditions not only differ from one another clinically but also at the biochemical level. The results of the present study further highlight this fact. Studies have demonstrated that the levels of PTX-3 are independent of systemic factors such as smoking status and HbA1c levels.[186] Hence, in such patients, PTX-3 could be used to provide better information as against other biomarkers that are affected by such conditions.

Studies at a greater scale, with larger sample size are required to further justify the results obtained in the present study. Further, this study was cross- sectional in nature and we did not evaluate the level of PTX-3 following treatment. Hence, interventional studies with various treatment modalities would shed further light on the variations in PTX-3

levels. It has been claimed that PTX-3 is produced locally in the tissues and that its levels in granulation tissue may serve as a better indicator of disease activity. Hence further studies that detect the PTX-3 levels in granulation tissue could provide more accurate results.

This study was performed to measure and compare the levels of PTX-3 among CP, AgP and H subjects. A total of 45 subjects who fulfilled the inclusion criteria [CP (n = 15), AgP (n = 15) and H (n = 15)] participated in this study. GCF samples were collected using microcapillary pipettes from sites with the maximum bleeding scores and the PTX-3 levels were estimated by means of ELISA.

From the results obtained in the present study, it can be inferred that:

i. Overall, the levels of PTX-3 in GCF were the highest in the AgP group followed by the CP group and were the least in the H group. The difference between the groups was found to be statistically significant.
ii. There was a statistically significant difference in the PTX-3 levels when the AgP and CP groups were compared.
iii. The levels of PTX-3 were greater in the periodontitis (CP and AgP) affected groups than in the H group and this difference was statistically significant.
iv. On the whole, a positive correlation was observed between the clinical periodontal parameters and PTX-3 levels in GCF of the study subjects.
v. The correlation was found to be statistically significant for all the clinical periodontal parameters except iPI.
vi. The strongest correlation was observed between PTX-3 and mSBI. This indicates that the levels of PTX-3 rise locally with increase in disease activity.

From the results obtained in the present study, we can conclude that, PTX-3 levels rise with an increase in the severity of the disease and can be used as a diagnostic tool to differentiate sites with active periodontal disease. The use of such biomarkers may

contribute to unravelling the changes occurring in the periodontium at the molecular level. Clinicians often encounter cases that are difficult to differentiate and classify. An understanding of the biochemical changes in periodontal health and various forms of disease, could provide valid answers towards arriving at an accurate diagnosis.

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